Microbiology Research Journal International

25(3): 1-7, 2018; Article no.MRJI.13349
ISSN: 2456-7043
(Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140)

Past & Current Status of Methicillin-Resistant *Staphylococcus aureus* & *Vancomycin-Resistant Staphylococcus aureus* in Pakistan

Azra Ayoub[1] and Atia Iqbal[1*]

[1]Department of Microbiology and Molecular Genetics, The Women University Multan, Pakistan.

Author's contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2018/13349
Editor(s):
(1) Tetsuya Akitsu, Professor, Division of Human, Environmental and Medical Engineering, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Japan.
(2) Luis Martinez-Sobrido, Assistant Professor, Department of Microbiology and Immunology, University of Rochester School of Medicine and Dentistry, USA.
Reviewers:
(1) Marcelo J. Mimica, Santa Casa School of Medicine, Brazil.
(2) Yukti Sharma, Hindu Rao Hospital, India.
(3) Zhitang Lu, College of Life Sciences, Hebei University, China.
Complete Peer review History: http://www.sciencedomain.org/review-history/27087

Review Article

Received 13 August 2014
Accepted 31 December 2014
Published 07 November 2018

ABSTRACT

Methicillin resistant *Staphylococcus aureus* (MRSA) has been emerging rapidly all over the world. Along with the developed countries, this agent has been spreading drastically among developing countries. Pakistan is one of these targeted countries where the spreading of MRSA is also a hot issue that is faced by the common people of Pakistan. Most of work has been done on the isolation of MRSA from the different patients in different cities of Pakistan. In article, the data showed the variable pattern of isolated MRSA among different cities. Rawalpindi was found to be most targeted area in 2007 to 2009 where MRSA prevalence was found 76% in Army Medical College Rawalpindi and this was gradually decreased up to 60.40% in 2011 to 2012 in five different hospitals of Rawalpindi. In Karachi, first case of MRSA was seen in 1989 (5%prevalence) then the prevalence of MRSA was increased (57%) in 2002 and then gradually decreased (43%) in 2004-2005 and (38.6%) in 2009. In Lahore, the MRSA prevalence was found 61% in 2000 to 2002 but it was decreased up to 38.6% in 2004, 27.77% (Jinnah Hospital Lahore) in 2007 to 2008 and 34.76% (Tertiary Care Hospital Lahore) in 2009. In 2012, the prevalence of MRSA was calculated 44% in two hospitals of Kohat. MRSA has become an alarming threat worldwide due to its resistance to multiple antibiotics.

**Corresponding author: E-mail: pisces_dream2000@yahoo.com;*

This condition is worst in developing countries due to non-sanitary conditions and unawareness of preventive measures taken by common people. The present study highlighted the previous and current pattern of MRSA in Pakistan from 1989 to 2013. Furthermore, the mechanism of MRSA and treatment were also being studied.

Keywords: Staphylococcus aureus; nosocomial infection; MRSA; VRSA; Pakistan.

1. INTRODUCTION

In the 21st century, different population of pathogenic bacteria have developed resistance to most of commercially available antibiotics and considered as major threat for human health. *Staphylococcus aureus* is one of the potential pathogen that caused large number of infections such as pneumonia, surgical site infections and skin and soft tissue infections [1]. Methicillin resistance in *Staphylococcus aureus* is produced by Penicillin Binding Protein – 2a (PBP2a) that is encoded by *mecA* gene which allows the organisms to grow and divide not only in the presence of methicillin but in other β-lactam antibiotics. The *mecA* gene is present on a mobile genetic element called *Staphylococcal* chromosome cassete *(SCC mec*). MRSA is endemic today in hospitals worldwide. It has been considered as danger for serious patients and for healthy persons also in general community [2]. In Pakistan, the current picture of MRSA is highly considerable as the lack of data impedes the true emergence of MRSA in Pakistan. Furthermore, the published data is also not enough to tell the exact scenario of MRSA in Pakistan. Analysis of published articles it further hinder the present situation of MRSA in Pakistan. This study make an attempt to collect and analyze the prevalence of MRSA in various cities of Pakistan that might be helpful to national and international organizations to recognize the present situation of this alarming bug in Pakistan.

1.1 History of Mrsa & Vrsa

Alexender Fleming in 1928 observed *Penicillium notatum* growth around *Staphylococci* [3]. So, Fleming discovered penicillin in 1940. Florey and Chain isolated and purified it [4]. Penicillin was introduced in 1943 when antibiotic resistance in *Staphylococci* was completely unknown. This fungal metabolite was used against *S. aureus* infections in the Second World War for the treatment of wounded soldiers of the Allied forces. Due to extensively use of penicillin in the Second World War, resistance of *S. aureus* against penicillin may have developed as early as 1946 [5]. Methicillin was introduced in human medicine in 1960s for the treatment of Penicillin's resistant *S. aureu's* mediated infections. However, first methicillin resistant *S. aureus* emerged in 1961 in England. The emergence of resistant strains was based on the usage of antimicrobial agents to treat *Staphylococcal* infections. MRSA has now become resistant to β-lactam and a number of antimicrobial agents [6]. The excess use antimicrobial agents becomes a trend and particularly pressing in the developing counties, where the MRSA is often the most probably causal agents in hospital acquired infections [7]. It is observed that MRSA prevalence varies between different hospitals in the same city in a country [8]. The development of antibiotic resistance is continuously increasing in developing countries because of unjustified use, over dosing without prescription and uncontrolled use of these antibiotics in agriculture, animal husbandry and fisheries [9]. MRSA strains have been associated with nosocomial or hospital acquired infections (HA-MRSA infections) world over and have emerged as an important cause of community acquired infections (CA-MRSA) as well in Pakistan [10,11,12]. The first MRSA case emerged in Pakistan in 1989 [11]. In May 1997 the center for diseases control and prevention (CDC) reported the first case of Vancomycin resistance in Japan. Strains of Vancomycin intermediate *Staphylococcus aureus* (VISA) with Vancomycin MIC of 8ug/ml have been documented form Japan, France and Germany [9]. In the early 1970's MRSA was found to be resistant to orally conventional antibiotics and sensitive only to emerge antibiotic Vancomycin. With the passage of time an increased resistance was found to Vancomycin in other species such as *Enterococcus* [13]. The emergence of Vancomycin resistant Enterococci gave rise to horizontal transmission by conjugation of resistance genes to *S. aureus.* Vancomycin resistance has been transferred from *Enterococcus facealis* to *S. aureus* through laboratory procedures that slowly express the phenotype [13]. The first case of Vancomycin resistant *S. aureus* (VRSA) strains was reported in two hospitals in the United States in 2002 that

showed high MIC of 32 ug/ml (Centers for disease control and prevention, 2002). In Pakistan, data was calculated about the VISA and VRSA is different in different cities of Pakistan. First time in 2004, VISA was reported as 4% of isolates in Lahore [14]. 13% VISA strains emerged in Karachi which is a matter of great concern [15], but Hakim et al. [15] & Kaleem et al. found that no *S. aureus* showed resistance against Vancomycin and linezolid in Rawalpindi. In 2009 one strain was found to be VRSA and four strains were found to be VISA [9]. This was first time Vancomycin resistance has been reported for Pakistan. In 2011, one isolate of *S. aureus* was detected as VISA [16]. Vancomycin and linezolid were found to be highly effective against MRSA [17]. The data which was collected from different cities of Pakistan showed divergent result that describes that prevalence of MRSA varies from one city to another in Pakistan (Table 1).

1.2 Mrsa Mechanism or Historical Research

MRSA organisms are in general resistant to different antibiotics including aminoglycosides, macrolides, flouroquinolones, clindamycin, trimethoprim/sulfamethaxozole, chloramphenicol and β-lactamases. This may occur due to spontaneous genetic mutation or involve acquirement of a genetic material through plasmid, transposon, integron or gene cassette [18]. The resistance may be due to attainment of a gene by horizontal transmission through conjugation of genetic material from *Staphylococcus sciuir* [13]. *mecA* gene is responsible for resistance to methicillin and other β-lactam antibiotics. After acquisition of *mecA,* the gene must be integrated and localized in the *S. aureus* chromosome. *mecA* encodes penicillin binding protein 2a (*PBP2a*) which differs from other penicillin binding proteins as its active site does not bind methicillin or others β-lactam antibiotics. As such *PBP2a* can continue to catalyze the transpeptidation reaction required for peptidoglycon cross linking enabling cell wall synthesis in the presence of antibiotics. As a consequence of the inability of *PBP2a* to interact β-lactam moieties, acquisition of *mecA* confers resistance to all β-lactam antibiotics in addition to methicillin [19]. For understanding of the methicillin resistance has led to the discovery of accessory factors that affect the level and nature of methicillin resistance. Accessory factors, such as fem factors, provide new possible targets while compounds that modulate methicillin resistant such as epicatechin gallate, derived from green tea and corilagin provide possible lead compounds for development of inhibitors [19].

1.3 Mrsa Prevalence in Pakistan

Data about MRSA prevalence is scarce due to paucity of data owing to lacunae in knowledge and variability in lab facilities among various hospitals. It has also been observed that frequency of prevalence of MRSA varying between 2 to 61% in the major cities of Pakistan [16]. Small cities have large population and congested social setup as compared to the large cities. The first incidence (5%) of MRSA occurred in Karachi in 1989 on the basis of provided data (Table 1). It was increased up to 57% in 2002. In 2004-05, the prevalence was decreased up to 43%. It seemed to be decreased from 2002-05, there might be some reasons. Firstly, the available data was collected from different sources because the number of isolated MRSA strains is different in different collected samples for example urine, pus etc. Secondly, the analyzation of prevalence of MRSA was done in different areas at the same time and in the same city by different researchers. Some areas have good sanitary conditions while others are not. In fact, nosocomial/community spreads of infections could be attributed to the lack of specific awareness among paramedical staff, and at least to the excess of genetic and physiological survival mechanisms of the chicaning multidrug resistant strains of pathogens. Horizontal transfer of resistant strains of pathogens to both community, outdoor and indoor hospital settings from surroundings is anticipated because of the crowding effect of patients and their attendants in developing countries. Further, in developed countries the horizontal transfer of pathogens from surroundings to hospitals would definitely be the minimum, but the possibility cannot be ruled out. Similar conditions are responsible for decrease and increase of MRSA prevalence in Rawalpindi and Lahore.

1.4 Treatment or Guideline

Before 1950, the treatment of MRSA was involved the Benzyl penicillin, a β-lactam antibiotic. In the late 1950s, *S. aureus* showed resistance to benzyl penicillin. Resistant strains typically produce an enzyme called a β-lactamase which is inactivated the β-lactam. In 1959 methicillin was synthesized that was resistant to β-lactamase hydrolysis. With the passage of time methicillin was used clinically and MRSA strains were isolated. Resistance was

due to acquiring of an additional *PBP2a* for another species. The glycopeptide antibiotic Vancomycin is the only option for antimicrobial therapy [19]. In Pakistan Vancomycin and linezolid are best remedy against MRSA mostly used in hospitals [33]. But now VRSA strains were isolated in last few years in Pakistan [9]. According to latest research (2013) it was observed that a specialized class of cells "*persistors*" are produced by all pathogens including MRSA. Survival is their only function. These cells prevent themselves form traditional antibiotic and undergo in a dormant state (Science daily, 2013). Lewis's team (2013) found that a drug called *ADEP* which change the dormant state into functional state then self destruction mechanism starts. The MRSA has ability to protect itself from the *ADEP.* Although it cannot completely destroy the cell but the *ADEP* resistant cells become wimpy. So, rifampicin or linezolid can easily work on persistor cells.

2. DISCUSSION

In this review article, the prevalence of MRSA was investigated in the light of previously published articles. The prevalence was analyzed in Pakistan by dividing the areas into East, West, North and South Pakistan. It was found that prevalence in north Pakistan was low in 1996 and increased at alarming rate in 2007-2009 then decreased in 2012 but still it remained upto 40% whereas in East Pakistan, an unusual trend was find out related to prevalence of MRSA. It was increased in 2000 but found 38.5% in 2002 but 61% in the same year but varies in different areas of East Pakistan. The reason could be the data which was collected from different areas showing the conditions of that particular area as compared to the prevalence of other areas. In Southern Pakistan, the prevalence of MRSA was found 57% very high in 2002. Compared to the prevalence of other areas. In Southern Pakistan, the prevalence of MRSA was found 57% very high in 2002. The data collected in next years 2005, 2009 & 2011 the prevalence of MRSA was found upto 43% 38.6% & 36.5% that was still considered high. The data related to prevalence of MRSA in West Pakistan is very limited, but still an alarming increase of MRSA was found in that area. Overall, the prevalence of MRSA from collected data showed an insight that how alarming this bug is going to penetrate in our societies that would be a great threat for Pakistani population.

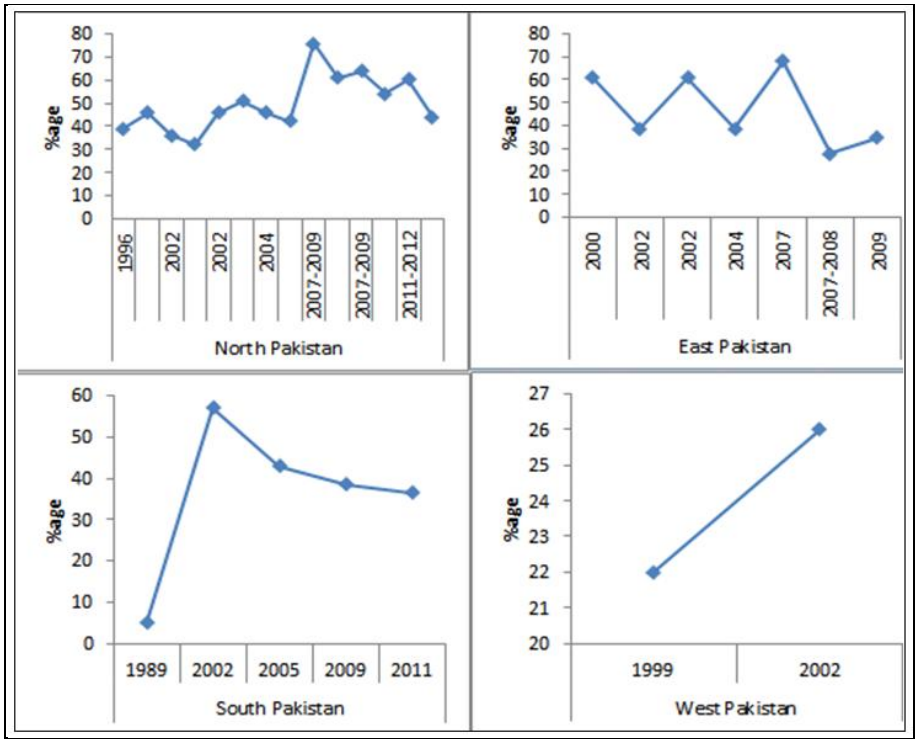

Fig. 1. MRSA prevalence in different poles of Pakistan

Table 1. The prevalence of MRSA in different cities of Pakistan

Study Period	MRSA Prevalence %	Cities	References
1989	5.0	Karachi	[11]
1996	39	Rawalpindi	[20]
1999	22.0	Sargodha	[21]
2000	61	Lahore	[22]
2000	29		[23]
2002	38.5	Mayo Hospital Lahore	[24]
2002	61	Lahore	[10]
2002	57	Karachi	[10]
2002	46	Rawalpindi	[10]
2002	46	Islamabad	[10]
2002	36	Peshawar	[10]
2002	32	Azad Kashmir	[10]
2002	26	Quetta	[10]
2003	65		[25]
2003	51	Rawalpindi	[20]
2004	46	Rawalpindi	[26]
2004	38.6	Lahore	[14]
2005	42.01	Rawalpindi	[27]
2004-2005	43.0	Karachi	[28]
2007	68	Gujranwala	[29]
2007-2008	27.77	JHL (Lahore)	[30]
2007-2009	76	AMC Rawalpindi	[31]
2007-2009	61	PIMS (Islamabad)	[31]
2007-2009	64	(Khyber Hospital Peshawar	[31]
2009	38.6	ZH (Karachi)	[9]
2009	34.76	TCH (Lahore)	[21]
2011	54	Peshawar	[32]
2011	36.5	Hyderabad	[16]
2011-2012	60.40	Five Hospital in Rawalpindi	[6]
2012	44	Two Hospitals of Kohat	[33]

MRSA has also been reported in the other developed as well as developing countries and their prevalence is reported to be increasing exponentially. Epidemiological data of HA-MRSA from separate studies shows that the highest rates (>50%) reported in Asia, North & South America and Malta. Intermediate rates (25–50%) are reported in Australia, China, Africa and some European countries [e.g. Portugal (49%), Greece (40%), Italy (37%) and Romania (34%)]. Other European countries have generally low prevalence rates (e.g. The Netherlands and Scandinavia) [34-37].

MRSA has become a challenge as a nosocomial pathogen in the United Kingdom. There has been a manifest increase in the prevalence of MRSA in India. Bangladesh is at risk to develop CA-MRSA due to absurd use of antibiotics [38]. The frequency of MRSA differs greatly from one city to another and among hospital in the same city but the data on the prevalence of MRSA strains in Pakistan is limited [9]. In developing countries like Pakistan antibiotic resistant bacteria remain a challenge and difficult to control because of unjustified use of antibiotics [33]. The present data is helpful to understand the current situation regradly MRSA Spread and provide an insight to these factors that are responsible for this by spreading in Pakistan. In this article, we review the prevalence and analyze the percentage of MRSA and VRSA in major cities of Pakistan.

3. CONCLUSION

The data showed an increase number of MRSA cases with the passage of time that might be an alarm to the Pakistani population. For the accurate estimation of MRSA incidence and prevalence need appropriate awareness and research work or survey in the whole Pakistan that require proper lab facilities and money.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. De San N, Densio O, Gasasria MF, De Mendonca R, Nonhoff C, Struelens MJ. Controlled evaluation of the IDI-MRSA

assay for detection of colonization by methicillin-resistant *Staphylococcus aureus* in diverse mucocutaneous specimens. J Clin Microbial. 2007;45(4):1098-1001.

2. John FM, Birgitte DeB, Truls ML, Aina EFM. MRSA is increasing in Norway: A time series analysis of reported MRSA and methicillin sensitive *S. aureus* cases. 1997-2010; 2013.
   DOI: 10.1371/journal.Pone.0070499
3. Fleming A. On the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of B. Influenza. Br J Exp Pathol. 1929;10:226-236.
4. Goldsworthy P, McFarlin AC, Florey H. Alexander Fleming and fairy tale of penicillin. Med J Aust. 2002;176:178-180.
5. Palumbi SR. Humans as world's greatest evolutionary force. Science. 2001;293: 1786.
6. Perveen I, Majid A, Kanwal S, Naz I, Sehar S, Ahmad S, Raza MA. Prevalance and antimicrobial suscpetitbiilty pattern of methicillin resistance *Staphylococcus aureus* and coagulase-negative in Rawalpindi, Pakistan. Br J of Medicine & Medical Research. 2013; 3(1):1998-2009.
7. Bukhari SZ, Ahmad S, Zia N. Antimicrobial susceptibility pattern of *Staphylococcus aureus* on clinical isolated and efficacy of labortary test to diagnose MRSA. J Ayub Med Coll Abbottabad. 2011;23(1).
8. Moniri R, Musay GA, Fadavi N. The prevalence of nasal carriage methicillin resistance *Satphylococcus aureus* in hospitalized patients. J Pak S Med sci. 2009;25(4):656-659.
9. Taj Y, Abdullah FE, Kazmi SA. Current pattern of antibiotic resistance in *Staphylococcus aureus,* clinical isolates and the emergence of vancomycin resistance. J of the College of Physi & Surg Pakistan. 2010;20(11):728-732.
10. Hafiz S, Hafiz AN, Ali L, Chugthai AS. Methicillin resistant *Staphylococcus aureus*: A multicentre study. J Pak Med Assoc. 2002;52:312-515.
11. Ashiq B, Tareen AK. Methicillin resistant *Staphylococcus aureus* in teaching hospital of Karachi-a labortary study. J Pak Med Assoc. 1989;39:6-9.
12. Zafar A, Stone M, Ibrahim S, Parveen Z, Hasan Z, Khan E, et al. Prevalent genotypes of methicillin-resistant *Staphylococcus aureus*: Report from Pakistan. J Med Med Microbial. 2011;60: 56-62.
13. Cookson B, Schmitz F, Fluit A. Introduction in Fluit AC, Schmitz FJ. (Eds), MRSA. Current prospectives. Caister Academic Press, Wymondham; 2003.
14. Bukhari MH, Iqbal A, Khatoon NA. Labortary study of susceptibility of methicillin-resistant *Staphylococcus aureus*. Pak J Med Sci. 2004;20:229-33.
15. Hakim ST, Arshad S, Iqbal M, Javaid SG. Vancomycin sensitivity of *Staphylococcus aureus* isolates from hospital patients in Karachi. J Libyan Med. 2007;764:177.
16. Bano S, Tunio SA, Mal S, JAAT AN. Frequency of methicillin resistance *Staphylococcus aureus* among isolates of wound infections from Hyderabad. J Sindh Uni Res (Sci.Ser). 2012;44(4):683-686.
17. Kaleem F, Usman J, Hassan A, Omair M, Khalid AR. Sensitivity pattern of Methicillin resistance *Staphylococcus aureus* isolated from patients admitted in tertiary care hospitals of Pakistan. IRAN J Microbial. 2010;2(3):141-143.
18. Mutto CA, Jenigan JA, Ostrowsky B, Ricket HM. Guideline for prevention of nosocomial transmission of multiresistant strains of *Staphylococcus aureus* and *Enterococcus*. J Infec control Hospital Epidemiol. 2003;24:362-8.
19. Paul DS, Peter WT. Methicillin resistance in *Staphylococcus aureus*. J Europe PMC funders Group. 2007;85(Pt 1):57-72.
20. Siddiqi GM, Siddique T, Anwar MS. Methicillin-resistant *Staphylococcus aureus*: A study in a tertiary care hospital. J Biomedical. 2009;25:56-8.
21. Ahmad A, Qureshi SF, Ansari N. Prevlence and antibiotic sensitivity pattern of methicillin resistant *Staphylococcus aureus* (MRSA) infection in a tertiary care hospital, Karachi: Liaquat National Hospital symposium; 2000.
22. Latif S, Anwar MS, Chaudhry NA. The susceptibility pattern of nosocomial Methicillin resistant *Staphylococcus aureus* (MRSA) isolates to vancomycin and other anti-*Staphylococcal* antibiotics. J Biomedical. 2002;16:32-5.
23. Khatoon N, Bukhari MH, Riaz JR, Sheikh AS, Iqbal A, Naeem S, et al. Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) infection labortary study at Mayo Hospital Lahore. J Biomed. 2002;18:42-52.
24. Safdar N, Narans L, Gordon B, Maki DG. Comparison of culture screening methods for detection of nasal carriage of methi-

cillin- resistant *Staphylococcus aureus*: A prospective study comparing methods. J Clin Microbial. 2003;41:3163-6.
25. Qureshi A, Rafi HS, Qureshi SM, Ali AM, The current susceptibility patterns of methicillin resistant *Staphylococcus aureus* to conventional and *Staphylococcus* anti-microbials at Rawalpindi. Pak J Med Sci. 2004;20(4):361-364.
26. Ali AM, Abbasi SA, Arif S, Mirza IA. Nosocomial infections due to methicillin-resistant *Staphylococcus aureus* in hospitalized patients. Pak J Med Sci. 2007; 23:593-96.
27. Perwaiz S, Barakzi Q, Farooqi BJ, Khursheed N, Sabir N. Antimicrobial susceptibility pattern of clinical isolates of methicilllin resistant *Staphylococcus aureus*. J Pak Med Assoc. 2007;57:2-4.
28. Ahmad K, Mahmood A, Ahmad MK. Methicillin resistant *Staphylococcus aureus* prevalence amongst community versus Hospital acquired skin and soft tissue infections. J Infect Dis Pak. 2007;16:14-6.
29. Ajmal AN, Mir F, Arslan M, Hafeez R, Attique R. Nosocomial methicillin-resistance *Staphylococcus aureus* frequency in a tertiaty care Hospital Lahore, Pakistan. J Biomedia. 2009;25. 2009/Bio-5:97-100.
30. Majeed Z, Arafat Y, Ajab Z, Malik SA, Abbasi WS, Ajab H. Genotype characterization of prevalent MRSA in Pakistan community hospitals. J ABCL. 2012;46(2):271-83.
31. Shafiq R, Shahina M, Ahmad JM, Syed HS, Malal R. Incidence of methicillin resistant *Staphylococcus aureus* in Peshawar. J. Ayub Med. College Abbotabad. 2011;23:1.
32. Yousaf MA, Sattar A, Habib ZH, Roy S. Clinical and diagnostic significance of methicillin-resistant *Staphylococcus aureus* infection in Bangladesh: A Systematic Review. J BMR. 2014;4(7):785-797.
33. Hussain M, Basit A, Khan A, Rahim K, Javed A, Junaid A, et al. Antimicrobial sensitivity pattern of methicillin resistant *Staphylococcus aureus* isolated from Hospitals of Kohat District, Pakistan. J of infec & Mole Bio. 2013;1(1):13-16.
34. Grundmann H, Aanensen DM, vanden Wijngaard CC, Spratt BG, Harmsen D, Friedrich AW. Geographic distribution of *Staphylococcus aureus* causing inva-sive infections in Europe: A molecular-epidemiological analysis. PLoS Med. 2010; 7:e1000215.
35. Song JH, Hsueh PR, Chung DR, Ko KS, Kang CI, Peck KR, et al. Spread of Methicillin-resistant *Staphylococcus aureus* between the community and the hospitals in Asian countries: An ANSORP study. J Antimicrob Chemother. 2011;66:1061– 9.
36. European centre for disease prevention and control. Antimicrobial resistance surveillance in Europe 2009. Annual report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Stockholm, Sweden: ECDC; 2010.
37. Mejía C, Zurita J, Guzmán-Blanco M. Epidemiology and surveillance of methicillin-resistant *Staphylococcus aureus* in Latin America. Braz J Infect Dis. 2010; 14(Suppl 2):S79–86.
38. Yousaf MA, Sattar A, Habib ZH, Roy S. Clinical and diagnostic significance of methicillin-resistant *Staphylococcus aureus* infection in Bangladesh: A systematic review. J BMR. 2014;4(7):785-797.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/27087