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Genotyping of Methicillin-resistant *Staphylococcus aureus* with rep-PCR

Aasma Akhtar[1], Naima Mahreen[1], Javaria Altaf[2], Muhammad Saqib[3], Mian Abdul Hafeez[4], Faisal Saeed Awan[*1]

[1]*Centre of Agricultural Biochemistry and Biotechnology, University of Agriculture, Faisalabad, Pakistan*

[2]*Department of Zoology, Government College University, Faisalabad, Pakistan*

[3]*Department of Clinical Medicine and Surgery, University of Agriculture Faisalabad, Pakistan*

[4]*Institute of Biochemistry and Biotechnology, University of Veterinary & Animal Sciences, Lahore, Pakistan*

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Abstract

Methicillin resistant Staphylococcus aureus (MRSA) is a significant infectious agent which cause infections in human and animals. This study was aimed to genotype human MRSA isolates recovered from wounds. Of the 66 wound cultures, 32 were confirmed as *S. aureus* and 23 had given positive signal MRSA, respectively. There were consistent banding patterns among all 23 MRSA isolates. The dendrogram attained from the Rep and BOX analysis showed 2 major groups showing a little bit maximum and distant relationship among MRSA strains. Henceforth, this technique could be of valuable use in scheming the modes as well as sources of transmission and mainly inhibiting the hospital related infections those are caused by the MRSA strains.

* **Corresponding Author:** Faisal Saeed Awan ✉ faisal.saeed@uaf.edu.pk

Introduction

Staphylococcus (S.) aureus is one of the most noteworthy pathogens causes wide range of infections both in human and animals (Trindade, 2003; Manga and Vyletělová, 2012; Grema *et al*., 2015). Being a well-armed pathogen, S. aureus harbors multiple toxins, virulence factors and resistant to variety of antimicrobials (Gorden and Lowy, 2008). About 20-40% of the human populations are carriers and some humans are midway carriers while others are constant carries (Foster, 2009; Williams, 1963).

Methicillin- is associated with the presence of *mec A* gene, which has resulted in resistance to almost all beta-lactam antimicrobials including penicillins, carbapenems, monobactams and cephalosporins (Kwon *et al*., 2006). In case of methicillin-susceptible *S. aureus* (MSSA), beta-lactam antimicrobials bind to PBPs, as a result of which synthesis of peptidoglycan layer disrupts, consequently inhibits the survival of *S. aureus*. On the other hand, in MRSA, the Mec A gene refers to the production of an altered PBP and PBP2a, to which beta-lactam antimicrobials are not capable to bind thus cannot disrupt the synthesis of peptidoglycan, facilitating the survival and growth of MRSA (Berger-Bachi, 2002).

The first report on methicillin resistance *S. aureus* was published in 1961 (Jevons *et al*., 1961), and this resistance is constantly rising all over the world. Methicillin resistant *S. aureus* are more virulent and appears to involve in chronic and serious infections than methicillin susceptible strains (Melzer *et al*., 2003; Wang *et al*., 2012). It is the fact that methicillin resistance is certainly associated to the importance of MecA gene, this gene codes PBP 2a protein which binds penicillin making it able to create molecular test for exact proof of MRSA (Peacock and Paterson, 2015). Due to high degree of genetic homology among MRSA strains has stopped the commonly used of genotyping methods.

For the molecular typing of methicillin resistant and non-resistant *S. aureus,* a variety of typing methods including arbitrarily primed polymerase chain reaction (AP-PCR), pulsed-field gel electrophoresis (PFGE), variable number tandem repeat analysis (VNTR) typing, multiple-locus variable-number tandem repeat analysis (MLVA), multilocus sequence typing (MLST) and *Staphylococcal* Cassette Chromosome *mec (*SCC*mec*) typing have been used (Sabat *et al*., 2003; Trindade *et al*., 2003; Stefani *et al*., 2012).

A BOX repetitive sequence is a greatly preserved repetitive DNA element that has been recognized in the *Streptococcus pneumoniae* chromosome. Even though the role of this constituent has not until now been entirely understood, it has been verified that the occurrence of a BOX sequence is linked with difference in colony cloudiness of the bacteria (Saluja and Weiser, 1995). BOX-PCR has been efficiently used for typing *S. aureus* along with other bacterial species (van Belkum *et al*., 1996).

All bacterial genome have repetitive elements sequences. These elements, which are spread throughout the chromosome, have been illustrated in comprehensive studies concerning repetitive sequences (Rep) (van Leeuwen, 2003; Stepan *et al*., 2004). These sequences are conserved by most bacteria and can be targeted, by the use of PCR, for epidemiological studies (Trindade *et al*., 2003). Rep-PCR is an uncomplicated PCR-based practice that basically targets various replicas of repetitive elements in the bacterial genome to produce DNA fingerprints (Versalovic *et al*., 1991). Generally, Rep-PCR has high-quality discriminatory power and its reproducibility is good as compare to other methods such as RAPD and PFGE (Manga and Vyletělová, 2012). The Rep-PCR with the combination of fluorophore-enhanced Rep-PCR (FERP) can also identify epidemic strains having MRSA background.

These methods provide rapid identification of epidemic strains which may concern with nosocomial infections (Versalovic *et al.,* 1994; Manga and Vyletělová, 2012; Aquadero *et al*., 2015). The present study was conducted with the aim to identify the genetic variation among Human MRSA strains isolated from different hospitals by using Rep primer.

Materials and methods

Sampling and Bacteriology

A total of 66 wound swabs were collected from human patients attending tertiary human hospitals in Faisalabad city. The swabs were processed for culturing by concentration technique (cefoxitin broth) and plated on CHROMagar™ MRSA. The presumptively identified staphylococcal growths were confirmed as *S. aureus* and MRSA, using Prolex, Staph Latex kit (Pro-lab Diagnostic, UK) and PBP2′ Test (Oxoid, UK). *Staphylococcus aureus*/MRSA were subjected to genomic confirmation.

DNA extraction

The samples were processed for genomic DNA extraction using Gene JET Genomic DNA Purification Kit by following the manual instructions of the kit (Thermo Scientific). The extracted genomic DNA was quantified by using.

Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, Dalware). The quality of DNA was checked by running all DNA samples on 0.8% gel electrophoresis and the working dilutions of all DNA samples of 10ng/µL were made using the stock DNA concentration for PCR analysis.

Molecular diagnosis of S. aureus and MRSA

The isolates were tested for the chromosomal genes 'Nuc' encoding (extracellular thermo-stable nuclease/thermo-nuclease/TNase, specific for *S. aureus*) and Mec A (methicillin resistant) by PCR (Brakstad *et al.*, 1992). The primers used were Mec A1: 5' GTA GAA ATG ACT GAA CGT CCG ATAA3', Mec A2: 5'-CCA ATT CCA CAT TGT TTC GGTCTA A-3', Nuc 5'- GCG ATT GAT GGT GAT ACG GTT-3' and Nuc -5'A GC CAA GCC TTG ACG AAC TAA AGC-3'. A Rep-PCR assay was carried out as described by van Belkum *et al.* (1995). For the Rep-PCR assay.

Approximately 15ng chromosomal DNA was used per reaction. Sequence of Rep primer was 5'-TCG CTCA AAA CAA CGA CAC C-3'. A BOX-PCR assay was carried out as described by van Belkum *et al.* (1995).

For the BOX-PCR assay, approximately 10ng chromosomal DNA was analyzed using Box primer: 5′-CTACGGCAAG GCGACGCTGACG-3.

Statistical analysis

The clear and visible bands obtained from BOX primer were scored using binary coded system as "1" for the presence of band in MRSA strain and "0" for the absence of band in MRSA strain in Microsoft Excel software. Dendrogram was generated by the Unweighted Paired Group Method with Arithmetic mean (Dendro UPGMA) average clustering.

In case of Rep primer, the acquired data were normalized using the unweighted pair-group method using arithmetic averages (UPGMA) clustering method with the Pearson correlation coefficient was utilized for the results interpretation and dendrogram construction.

Results

A total of 66 samples (Table 1) were collected from patients attending tertiary human hospitals in Faisalabad city and confirmation of *S. aureus* and MRSA was done through PCR using Nuc and MecA primers respectively. The 'Nuc' gene encodes (extracellular thermo-nuclease or TNase, specific for *S. aureus*) and mecA is methicillin resistant by PCR. Out of 66 samples 'Nuc' gene was confirmed in 32 samples and 'MecA' gene was confirmed in 23 samples (Table-1).

The product size of 'nuc' fragment was 279 bp and of 'Mec*A*' fragment was 310 bp. Genetic characterization of 23 MRSA samples out of 66 was analyzed by using Rep primer. The results showed reliable banding pattern (Fig.-1). Total of 177 fragments were amplified by Rep primer.

The size of bands range from 100-1000 bp. Maximum no. of bands shown by the sample no. 19 while minimum has shown by the sample no. 21 and 6. Out of 66 samples 23 samples were used for genetic characterization of MRSA isolates by using BOX primer.

All MRSA strains show reliable banding pattern (Fig.-2). Total 115 bands were amplified by BOX primer. The size of the bands ranging from 250-3000 bp.

Table 1. Confirmation of MRSA strains using Nuc and Mec A PCR methodology.

Sample I.D	Sources	Nuc (–ve/+ve)	Mec A (ve/+ve)
5	Allied hospital Male-43 yrs Laparotomy (surgery)	-ve	-ve
6	Allied hospital Male-48 yrs Burn wound	+ve	-ve
7	Allied hospital Male-50 yrs Gangrene (surgery)	+ve	-ve
8	Allied hospital Male-29 yrs Esophagotomy (surgery)	-ve	-ve
9	Allied hospital Female-30 yrs Surgical wound	+ve	-ve
10	Allied hospital Female-70 yrs Gangrene in leg	+ve	-ve
11	Allied hospital Male-44 yrs Burn wound	+ve	-ve
12	Allied hospital Male- 4yrs Burn	+ve	-ve
16	Allied hospital Female-42 yrs Burn	+ve	+ve
17	Allied hospital Male-14 yrs Burn	+ve	-ve
18	Allied hospital Male-18 yrs Burn	+ve	-ve
31	Allied hospital Male-18 yrs Burn	+ve	-ve
32	Allied hospital Male-55 yrs Surgery	-ve	-ve
34	Allied hospital Male- 46 yrs Burn	+ve	-ve
35	Allied hospital Male-31yrs Appendix surgery	-ve	+ve
39	Allied hospital Female-45 yrs Surgical wound	-ve	-ve
40	Allied hospital Male-32 Burn	-ve	+ve
41	Allied hospital Female-40 yrs Burn	-ve	-ve
52	Allied hospital Male-44 yrs Burn	-ve	-ve
54	Allied hospital Male-21 yrs Surgical wound	-ve	-ve
55	Allied hospital Male-26 yrs Surgical wound	-ve	-ve
56	Allied hospital Male-45 yrs Diabetic foot	-ve	-ve
58	Allied hospital Female-38 yrs leprotomy	-ve	-ve
60	Allied hospital Male-49 yrs Surgery (appendix)	-ve	-ve
61	Allied hospital Male-53 yrs Surgery(appendix)	-ve	-ve
63	Allied hospital Male-35 yrs Surgery (cellulitis pos inj.)	-ve	-ve
67	Allied hospital Male-62 yrs Burn	-ve	-ve
68	Allied hospital Female-36yrs Burn	-ve	-ve
72	Allied hospital Male-34 yrs Surgery	-ve	-ve
75	DHQ Hospital Female-25 yrs Burn	-ve	+ve
76	DHQ Hospital Male-28 yrs Leg surgery	-ve	-ve
77	DHQ Hospital Male-44 yrs Diabetic foot	-ve	-ve
80	National Hospital Male-52 yrs Surgery (gangrene)	-ve	-ve
82	National Hospital Male-28 yrs Burn	-ve	-ve
85	DHQ Hospital Male- 35 yrs Burn	-ve	-ve
86	DHQ Hospital Male- 38 yrs Leprotomy	-ve	-ve
88	DHQ Hospital Male- 55 yrs Chest surgery	+ve	+ve
89	DHQ Hospital Female-32 yrs Burn	+ve	-ve
91	DHQ Hospital Male- 46 yrs Surgery	+ve	+ve
92	DHQ Hospital Male- 46 yrs Surgery	-ve	-ve
95	DHQ Hospital Male- 61 yrs Diabetic foot	+ve	+ve
97	DHQ Hospital Female- 26 yrs Appendix (surgery)	+ve	+ve
98	DHQ Hospital Female- 24 yrs Burn	-ve	+ve
99	DHQ Hospital Female- 43 yrs Surgery	-ve	-ve
102	National Hospital Male- 23 yrs Surgery	-ve	-ve
106	National Hospital Male- 42 yrs Burn	-ve	-ve
107	National Hospital Male-18 yrs Burn	-ve	-ve
111	Allied Hospital Female-38 yrs Burn	-ve	-ve
115	Allied Hospital Male-56 yrs Burn	-ve	-ve
121	Allied Hospital Male-23 yrs Surgery	+ve	-ve
122	DHQ Male-24 yrs Burn	+ve	+ve
123	National Hospital Female-28 yrs Burn	+ve	+ve
124	Allied Hospital Male-40 yrs Burn	+ve	-ve
126	DHQ Male-55 yrs Diabetic foot	+ve	+ve
133	Allied Hospital Female-11 yrs Burn	+ve	+ve
134	Allied Hospital Male-24 yrs Burn	-ve	+ve
136	Allied Hospital Male- 36 yrs Burn	+ve	+ve

Sample I.D	Sources	Nuc (–ve/+ve)	Mec A (ve/+ve)
137	DHQ Female- 20 yrs Burn	+ve	+ve
138	DHQ Male-28 yrs Burn	+ve	+ve
139	DHQ Female-15 yrs Burn	+ve	+ve
141	DHQ Female-21 yrs Surgery	+ve	+ve
143	Allied Hospital Female-20 yrs Surgery	+ve	+ve
144	DHQ Female-32 yrs Surgery	+ve	+ve
145	DHQ Male- 30 yrs Burn	+ve	+ve
146	Allied Hospital Male- 51 yrs Surgery	+ve	+ve
148	Allied Hospital Female-21 yrs Burn	+ve	-ve

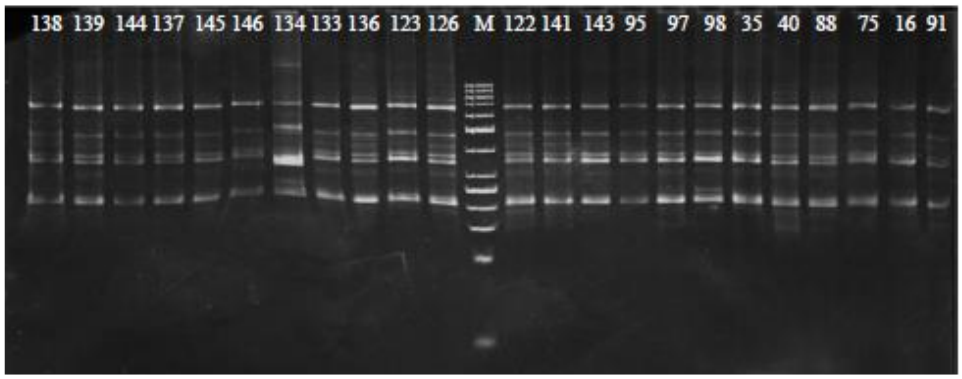

Fig. 1. Rep (PCR) amplification of MRSA using Rep primer. M is 50bp Ladder.

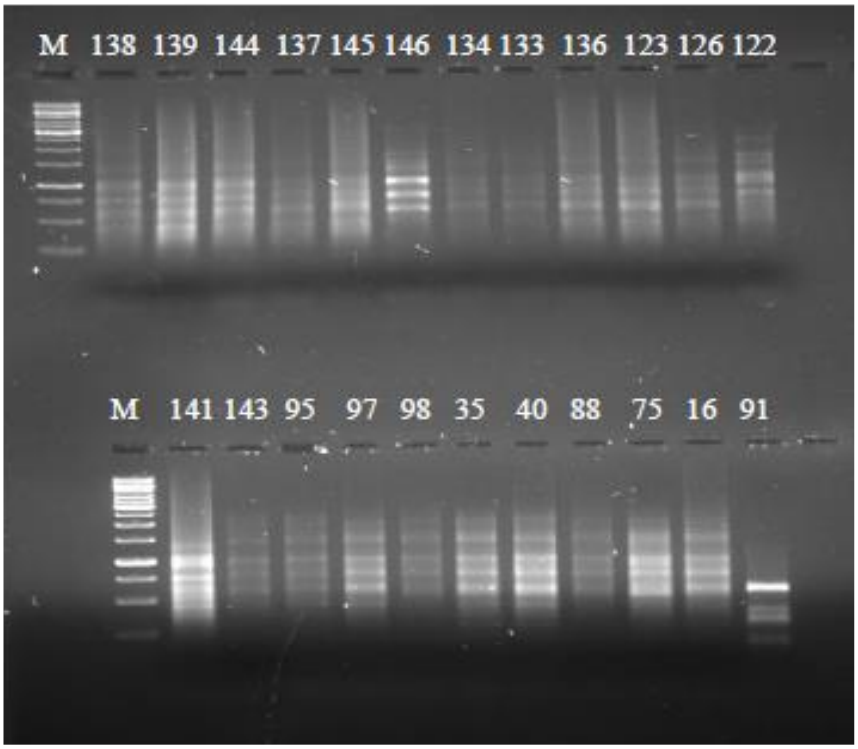

Fig. 2. BOX (PCR) of MRSA concurrence using BOX primer. M is a 1 kb Ladder.

Genetic relationship among MRSA samples

Genetic relationship between MRSA isolates was identified through cluster analysis by Unweighted Paired Group of Arithmetic Means Average (UPGMA) method using Dendro UPGMA software for BOX and UPGMA, Pearson coefficient for Rep. In Rep-PCR all 23 samples were clustered into two main clusters A and B (Fig. 3). Cluster B contains one member 134 which show distant relationship in comparison to other members and cluster A subdivided into two sub

clusters C and D. Cluster C further subdivided into cluster E and F and cluster D subdivided into sub cluster G which contains two closely related samples 91 and 138 and sub cluster H which contains three members 144, 139 and 137 in which sample 144 and 139 show maximum genetic relationship. Cluster F subdivided into two groups I which contains three members 16, 35 and 75 from which 16 and 35 are closely related to each other and sub cluster J contains two closely related members 133 and 146. Sub cluster E further subdivided into two clusters K and L in which cluster L contains two members 126 and 145 which are closely related to each other and cluster K further subdivided into two sub clusters M and N in which N contain only one member 136 which linked with M but show distant relationship and cluster M further subdivide into two clusters O and P from which cluster P contains two members 98 and 123 which show maximum genetic relationship and cluster O further divided into two clusters Q and R in which cluster Q contains two closely related members 88 and 40 and R contains five members 97, 141, 143, 95 and 122 from which 97 and 141 show maximum genetic relationship.

In case of BOX-PCR all the 23 samples were clustered into two main groups. First group consists of 17 members while second group contains 6 members (Fig. 4). In first group, maximum genetic relationship was found among 122, 136 and 134, 146 and 122, 126 and 143, 95 and in second group, samples 138 and 139 show maximum genetic relationship while sample 91 and 141 show distant relationship in comparison of other members of same group, respectively.

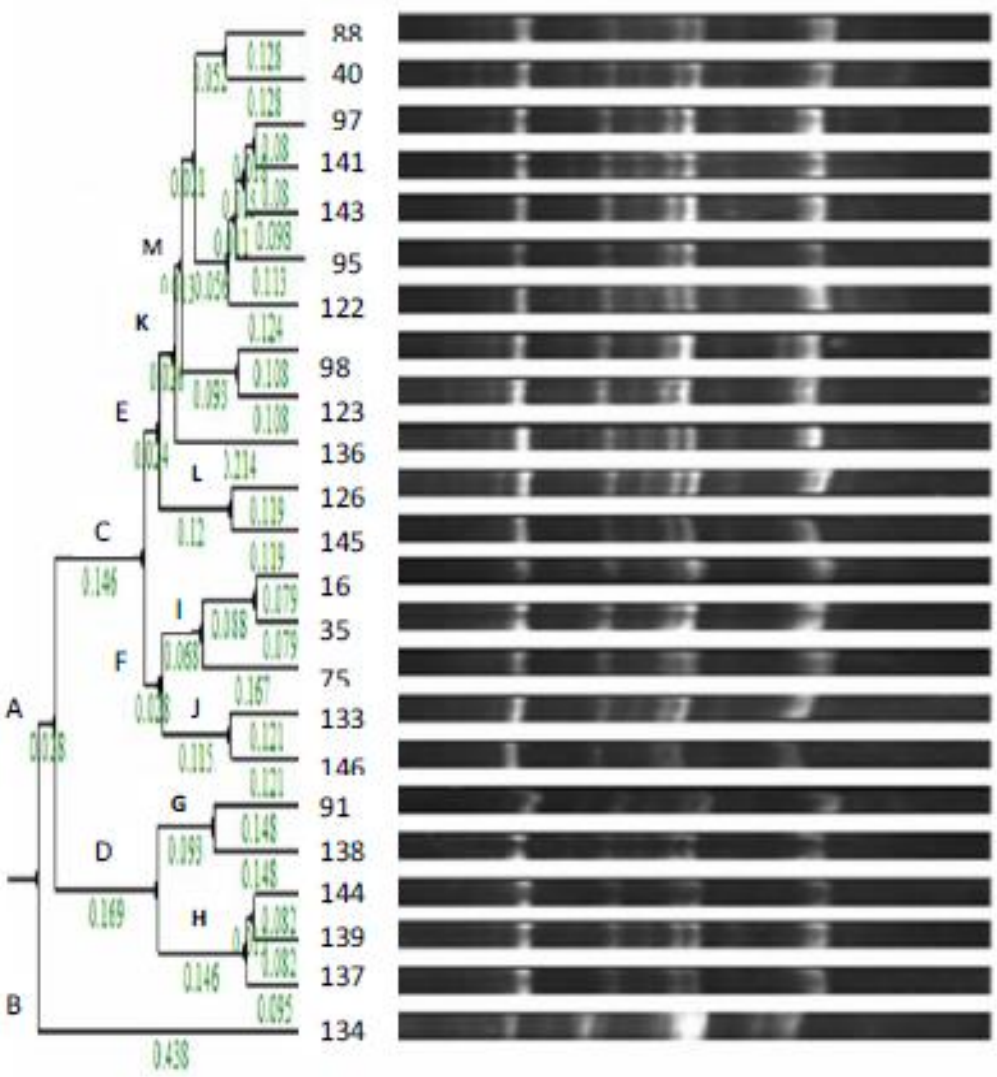

Fig. 3. Dendrogram of Rep-PCR using the UPGMA clustering, Pearson method.

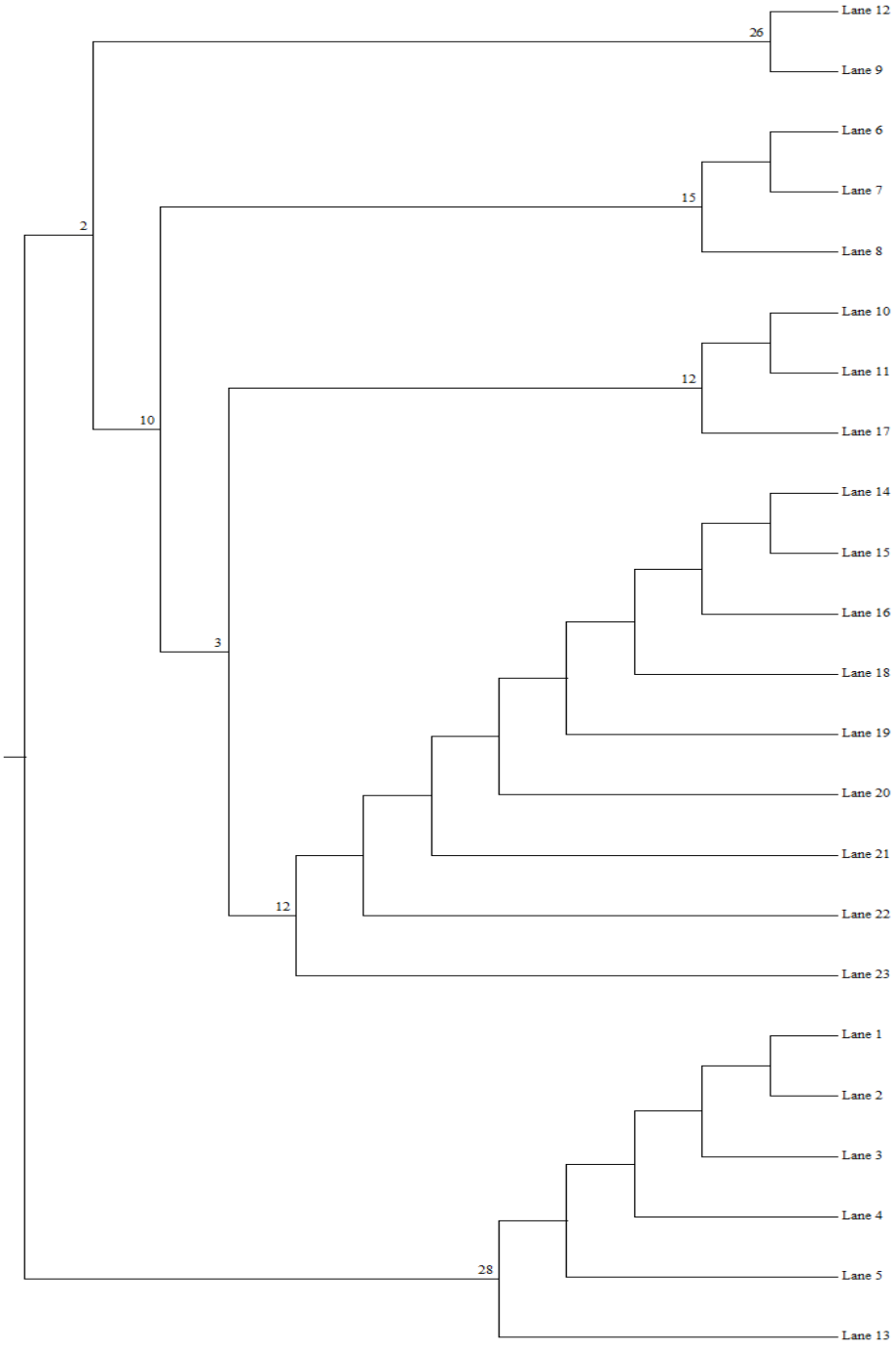

Fig. 4. Dendrogram of BOX-PCR using the Dendro UPGMA

Discussion

Staphylococcus aureus is a member of the family Staphylococcaceae (Firmicutes), Gram-positive facultative anaerobic bacteria that exhibits a coccal morphology, and are non-spore forming and non-motile in nature. *S. aureus* is well thought-out as one of the most notable pathogen causing infections in human and animals.

Methicillin-resistant *S. aureus* (MRSA) is placed amongst the most important and widespread pathogens having resistant to multiple antibiotics all around the world. In 1959, methicillin was introduced to treat infections caused by *S. aureus* resistance towards penicillin (Enright *et al*., 2002).

In 1961, just about two years after the introduction of methicillin, the first *S. aureus* isolate containing resistance to methicillin was reported (Jevons *et al*., 1961). In case of MRSA, the mec A gene refers to the production of an altered Protein Binding Protein (PBP) and PBP2a, to which beta-lactam antimicrobials are not able to bind therefore cannot disrupt the synthesis of peptidoglycan, assisting the survival and growth of MRSA (Berger-Bachi, 2002). In present study, percentage of total *S. aureus* and MRSA (n=66) was found to be 51.5% and 34.8% respectively. Various typing techniques are available for the discrimination of *S. aureus* strains, which can be practiced as very useful tools by both the epidemiologist as well as the clinician. Numerous genotypic techniques have been practiced in the last few decades. Initially, these techniques were only used by a small number of research laboratories; however they have been progressively more in use in clinical practices (Maslow *et al*., 1993). In the last decade, several molecular techniques have been developed for the typing of *S. aureus* strains, including arbitrarily primed polymerase chain reaction (AP-PCR), pulsed-field gel electrophoresis (PFGE), variable number tandem repeat analysis (VNTR) typing, multiple-locus variable-number tandem repeat analysis (MLVA), and PCR based methods (Lee *et al*., 2011; Del Vecchio *et al*., 1995; van der Zee *et al*., 1999). But the most common genotyping methods include Rep and BOX PCR. These techniques have been used for the discrimination of isolates of several bacterial species together with *S. aureus* (Swami Nathan and Matar, 1993; Farber, 1996; Jeršek *et al*., 1996). Rep-PCR was illustrated by Versalovic *et al.* (1991) for the study of the bacterial genome by means of examination of specific conventional profiles for strains acquired by PCR amplification of repetitive DNA sequence present inside the bacterial genome. Usually, Rep-PCR has high-quality discriminatory power and its reproducibility is good as compare to other techniques such as RAPD and PFGE. The Rep-PCR with the combination of fluorophore-enhanced Rep-PCR (FERP) can also identify endemic strains having MRSA background (Versalovic *et al.,* 1994).

A BOX repetitive sequence is a greatly preserved repetitive DNA element that has been recognized in the *Streptococcus pneumoniae* chromosome. The exact role of BOX is still unknown but it has been confirmed that the occurrence of a BOX sequence is linked with difference in colony cloudiness of the bacteria (Saluja and Weiser, 1995). BOX-PCR has been efficiently used for typing *S. aureus* along with other bacterial species (van Belkum *et al*., 1996). BOX-PCR is basically a fastidious adaptation of Rep-PCR (Versalovic *et al*., 1991). that makes use of the BOX-A1R primer which was developed by Versalovic *et al.* (1994). These methods offer rapid recognition of epidemic strains which may be related with nosocomial infections. In present study it was found that 10ng/µl and 15ng/µl gave the best results and they were finally used as an optimized DNA concentration for BOX and Rep (PCR), respectively. Similarly 2.5mM dNTPs, 3mM MgCl2, 3µl template DNA and 1U *Taq* DNA polymerase was found optimum for both (PCR) in a total volume of 20µl reaction mixture. All the reactions were repeated thrice to check the stability of the BOX and Rep (PCR) amplification. The major proportion of MRSA (48.24%) was isolated from wounds (accidental and surgical) followed by abscesses (15.29%), burns (11.76 %), carbuncle (8.24%), diabetic foot and IV-catheter (7.06%) and urinary catheter (2.35%). Mahmood *et al*., (2010) found 28.68% MRSA isolates from IV catheters ETT suction tips whereas 24.53% MRSA from pus samples and also collected samples from ETT suctions alongside with IV-catheters and described combined results. Perwaiz and co-workers (2007) reported 32% positive MRSA isolates from pus samples and 36% positive MRSA isolates from wound samples. Van der Zee *et al*. (1999) performed a study and outcome found by Rep-PCR were assessed to that of other genotypic methods that had previously been used for the purpose of characterization of the strains. In present study two primers Rep and BOX were used to amplify reproducible PCR products of 23 MRSA samples. Both primers show reliable banding pattern. To check genetic relationship among MRSA isolates dendrogram were generated using clustering UPGMA method.

Rep-PCR showed a little bit complicated clustering as compare to BOX-PCR. In Rep-PCR, two main groups were found. Second group comprised of one member 134 is distantly related to other members and first group contained 22 samples. Maximum genetic relationship was found among 133, 146 and 16, 35 and 126, 145 and 98, 123 and 143, 141 and 88, 40. In BOX all the 23 samples were clustered into two main groups. First group consists of 17 members while second group contains 6 members. Maximum genetic similarity was shown by 146, 134 and 122, 136 and 133, 123 and 143, 95 in first group and 138, 139 in second group.

Likewise, a good reproducibility of Rep-PCR fingerprinting was proved by Kang and Dunne (2003). They established high stability of fingerprints obtained from DNA isolated from 24, 48 and 72 h old bacterial cultures and from 5, 10 and 15 successive sub cultured strains. Moreover, Yang and Yen (2012) reported that different concentration of template DNA, presence or absence of 7 bovine serum albumin on different annealing temperature and the growth phase of the culture template may not have notable effect on the BOX- fingerprints of E. coli of any origins. These results are quite comparable with the earlier findings of Rep and BOX analysis on MRSA isolates. The present research on MRSA is a unique of its kind as in Pakistan no previous report on MRSA genotyping with Rep and BOX analysis is present to show the genetic relationship.

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References

Aguadero V, González Velasco C, Vindel A, Gonzalez Velasco M, Moreno JJ. 2015. Evaluation of rep-PCR Diversi Lab versus PFGE and spa typing in genotyping methicillin-resistant Staphylococcus aureus (MRSA). Br J Biomed Sci 2015; **72(3),** 120-127.

Berger-Bachi B. 2002. Resistance mechanisms of gram-positive bacteria. Int. J. Med. Microbiol **292,** 27-35.

Bergey DH, Holt JG, Krieg NR, Sneath PH, Staley JT, Williams ST. 1994. Gram-positive cocci. In, Bergey's Manual of Determinative Bacteriology, 9th (Ed.). Williams and Wilkins (Eds.). Baltimore, Maryland USA.

Brakstad OG, Assbakk K. Maeland JA. 1992. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the *Nuc* gene. J. Clin. Microbiol **30,** 1600-1654.

Brown DF, Edwards DI, Hawkey PM, Morrison D, Ridgway GL, Towner KJ, Wren MW. 2005. Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant *Staphylococcus aureus* (MRSA). J. Antimicrob. Chemother **56,** 1000-18.

Chambers HF. 2001. The changing epidemiology of *Staphylococcus aureus*. Emerg. Infect. Dis **7,** 178-82.

Del Vecchio VG, Petroziello JM, Gress MJ, McCleskey FK, Melcher GP, Crouch HK, Lupski JR. 1995. Molecular genotyping of methicillin-resistant *Staphylococcus aureus* via fluorophore-enhanced repetitive-sequence PCR. J. Clin. Microbiol **33,** 2141-4.

Enright MC, Robinson DA, Randle G, Feil EJ, Grundmann H. Spratt BG. 2002. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). Proc. Natl. Acad. Sci. USA **99,** 7687-7692.

Farber JM. 1996. An introduction to the hows and whys of molecular typing. J. Food Prot **59,** 1091-101.

Ferry T, Perpoint T, Vandenesch F. Etienne J. 2005. Virulence determinants in *Staphylococcus aureus* and their involvement in clinical syndromes. Curr. Infect. Dis. Rep **7,** 420-8.

Foster TJ. 2009. Colonization and infection of the human host by *staphylococci*: adhesion, survival and immune evasion. Vet. Dermatol **20,** 456-70.

Fournier B. Philpott DJ. 2005. Recognition of *Staphylococcus aureus* by the innate immune system. Clin. Microbiol. Rev **18,** 521-40.

Gordon RJ, Lowy FD. 2008. Pathogenesis of methicillin-resistant *Staphylococcus aureus* infection. Clin Infect Dis. 2008 Jun **1,** 46. Suppl 5: S350-9. DOI: 10.1086/533591.

Grema HA, Geidam YA, Gadzama GB, Ameh JA, Suleiman A. 2015. Methicillin resistant *Staphyloccus aureus* (MRSA): A review. Adv. Anim. Vet. Sci **3(2),** 79-98.

Jeršek B, Tcherneva E, Rijpens N, Herman L. 1996. Repetitive element sequence-based PCR for species and strain discrimination in the genus Listeria. Lett. Appl. Microbiol **23,** 55-60.

Jevons MP. 1961. "Celbenin"- Resistant *Staphylococci.* BMJ **1,** 124-5.

Kang HP, Dunne M. 2003. Stability of repetitive sequence PCR patterns with respect to culture age and subculture frequency. J. Clin. Microbiol **41,** 2694-2696.

Kwon NH, Park KT, Moon JS, Jung WK, Kim SH, Kim JM, Hong SK, Koo HC, Joo YS, Park YH. 2006. Staphylococcal cassette chromosome mec (*SCCmec*) characterization and molecular analysis for methicillin-resistant *Staphylococcus aureus* and novel *SCC mec* subtype IVg isolated from bovine milk in Korea. J. Antimicrob. Chemother **56,** 624-632.

Lee CJ, Sankaran S, Mukherjee DV, Apa ZL, Hafer CA, Wright L, Larson EL, Lowy FD. 2011. *Staphylococcus aureus* or pharyngeal carriage in a prison population. Clin. Infect. Dis **52,** 775-778.

Lowy FD. 1998. *Staphylococcus aureus* infections. N. Engl. J. Med **339,** 520-32.

Mahmood K, Tahir T, Jameel T, Ziauddin A, Aslam HF. 2010. Prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) Causing Nosocomial Infection in a Tertiary Care Hospital. ANNALS **16,** 91-96.

Manga I, Vyletělová M. 2012. Rep-PCR-based typing as a tool for tracking of MRSA infection origin. Acta Univ. Agric. Silvic. Mendelianae Brun **60,** 251-256.

Marshall JH, Wilmoth GJ. 1981. Pigments of *Staphylococcus aureus*, a series of triterpenoid carotenoids. J. Bacteriol **147,** 900-13.

Maslow JN, Mulligan ME, Arbeit RD. 1993. "Molecular epidemiology: application of contemporary techniques to the typing of microorganisms." Clin. Infect. Diseases 153-162.

Melzer M, Eykyn SJ, Gransden WR, Chinn S. 2003. Is methicillin resistant *Staphylococcus aureus* more virulent than methicillin susceptible *S. aureus*. A comparative cohort study of British patients with nosocomial infection and bacteremia. Clin Infect Dis **37,** 1453–60. http://dx.doi.org/10.1086/379321.

Novick RP. 2006. *Staphylococcal* pathogenesis and pathogenicity factors: Genetics and regulation. In: Fischetti, R.P. J.J. Ferretti, D.A. Portnoy, J.I. Rood. (Eds.). Gram-Positive Pathogens. ASM Press, Washington, D.C 496-516.

Peacock SJ, Paterson GK. 2015. Mechanism of methicillin resistance in *Staphylococcus aureus*. Annual Rev. Biochem **84,** 577-601.

Perwaiz S, Barakzi Q, Farooqi BJ. 2007. Antimicrobial susceptibility pattern of clinical isolates of Methicillin resistant *Staphylococcus aureus*. J. Pak. Med. Assoc **57,** 2-4.

Sabat A, Krzyszton-Russjan J, Strzalka W, Filipek R, Kosowska K, Hryniewicz W, Travis J, Potempa J. 2003. New method for typing *Staphylococcus aureus* strains: multiple-locus variable-number tandem repeat analysis of polymorphism and genetic relationships of clinical isolates. J. Clin. Microbiol **41,** 1801-4.

Saluja SK, Weiser JN. 1995. The genetic basis of colony opacity in *Streptococcus pneumoniae*: evidence for the effect of box elements on the frequency of phenotypic variation. Mol. Microbiol **16(2),** 215-27. 0950-382.

Shu-Hua Wang, Yosef Khan, Lisa Hines, José R, Mediavilla, Liangfen Zhang, Liang Chen, Armando Hoet, Tammy Bannerman, Preeti Pancholi D, Ashley Robinson, Barry N, Kreiswirth, Kurt B, Stevenson. 2012. Methicillin-Resistant Staphylococcus aureus Sequence Type 239-III, Ohio, USA, 2007-2009. Emerg. Infect. Dis 2012 **18,** L 1557-1565,

Stefani S, Chung DR, Lindsay JA, Friedrich AW, Kearns AM, Westh H, Mackenzie FM. 2012. Meticillin-resistant *Staphylococcus aureus* (MRSA): global epidemiology and harmonization of typing methods. Int. J. Antimicrob. Agents **39,** 273-282.

Stepan J, Pantucek R, Doskar J. 2004. Molecular diagnostics of clinically important *staphylococci*. Folia Microbiol. (Praha) **49,** 353-86.

Swaminathan B, Matar GM. 1993. Molecular typing methods. In: Persing, D.H., T.F. Smith, F.C. Tenover and T.J. White (Eds.). Diagnostic molecular microbiology: principles and applications. Washington 26-50.

Trindade PA, McCulloch JA, Oliveira GA, Mamizuka EM. 2003. Molecular techniques for MRSA typing: current issues and perspectives. Braz. J. Infect. Dis **7,** 32-43.

Van Belkum A, Kluitmans J, van leeuwen W, Bax R, Quint W, Peters E, Fluit A, Vandenbroucke-Grauls C, van den Brule A, Koeleman H. 1995. Multicenter evaluation of arbitrarily primed PCR for typing of *Staphylococcus aureus* strains. J. Clin. Microbiol **33,** 1537-47.

Van Belkum A, Sluijuter M, de Groot R, Verbrugh H, Hermans PW. 1996. Novel BOX repeat PCR assay for high-resolution typing of *Streptococcus pneumoniae* strains. J. Clin. Microbiol **34(5),** 1176-9.0095-1137.

Van der Zee A, Verbakel H, van Zon JC, Frenay I, van Belkum A, Peeters M, Buiting A, Bergmans A. 1999. Molecular genotyping of *Staphylococcus aureus* strains: comparison of repetitive element sequence-based PCR with various typing methods and isolation of a novel epidemicity marker. J. Clin. Microbiol **37,** 342-9.

Van Leeuwen W. 2003. Molecular Approaches for Epidemiological Characterization of *Staphylococcus aureus*. pp 95-136. In. MRSA Current Perspectives. Fluit, A.C. and F.J. Schmitz (Eds.). Caister Academic Press, Norfolk England.

Versalovic J, Kapur V, Koeuth T, Mazurek GH, Whittam TS, Musser JA, Lupski JR. 1994. DNA fingerprinting of pathogenic bacteria by fluorophore-enhanced repetitive sequence-based polymerase chain reaction. Arch. Pathol. Lab. Med **119,** 240-246.

Versalovic J, Kapur V, Lupski JR. 1991. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nucleic Acids Res **19,** 6823-31.

Wieland B, Feil C, Gloria-Maercker E, Thumm G, Lechner M, Bravo JM, Poralla K, Gotz F. 1994. Genetic and biochemical analyses of the biosynthesis of the yellow carotenoid 4, 4'-diaponeurosporene of *Staphylococcus aureus*. J. Bacteriol **176,** 7719-26.

Williams R. 1963. Healthy carriage of *Staphylococcus aureus:* its prevalence and importance. Bacteriol. Rev **27,** 56-71.

Yang A, Yen C. 2012. PCR optimization of BOX-A1R PCR for microbial source tracking of *Escherichia coli* in waterways. J. Exp. Microbiol. Immunol **16,** 85-89.