Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 10, No. 6, p. 330-335, 2017
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Molecular genotyping of methicillin-resistant *Staphylococcus aureus* by employing DNA marker technology

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Article published on July 30, 2017

Key words: *Staphylococcus aureus*; Methicillin-resistant; Genetic diversity; RAPD.

Abstract

Staphylococcus aureus are Gram positive, non-motile and facultative anaerobic *coccus* often golden yellow cells with pigments and usually arranged in clusters like grapes. Methicillin-Resistant *Staphylococcus aureus* (MRSA) are *S. aureus* strains that are resistant to methicillin and many other antibiotics. The present study aimed to molecular characterizes the 23 MRSA isolates obtained from effected patients and characterized by Randomly Amplified Polymorphic DNA (RAPD). Total of 14 RAPD primers amplified 827 fragments with range of 3 to 8 and level of polymorphism was 93.9%. Genetic similarities range is 88.73% to 52.11%, maximum genetic similarity was found between strain number 143 and 136 while the minimum genetic similarity was found among strain number 141 and 88. Genetic relationship among the MRSA isolates was analyzed through cluster analysis. All isolates were clustered in three major groups and maximum isolates were present in the second group. The present findings could be of beneficial use in scheming the sources as well as modes of transmission, trailing the spread of strains within and between hospitals, and mainly inhibits the nosocomial infections that are caused by the MRSA.

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Introduction

Methicillin-Resistant *Staphylococcus aureus* (MRSA) in 1960s firstly identified in hospitals, reformed in 1990s community acquired. MRSA is antibiotic-resistant to pathogen (Chambers and Deleo, 2009; Kennedy and Deleo, 2009; Deleo *et al.*, 2010; Otter and French, 2010). Action of methicillin resistance is genetically regulated by SCCmec which is Staphylococcus Cassette-Chromosome (SCCmec). Hospital associated (HA-MRSA) have characteristic to form colonies. It cause many diseases in hospitalized persons that are already disposed to risk factors that include surgeries, individual having catheter like devices, an impaired immune state or before the exposure of antibiotic. It is usually isolated from wound injuries, blood stream infection and pneumonia linked to ventilator. The MRSA strains generally carry type i, ii, and iii of SCCmec and have resistant to multidrug (MDR) (Gordon and Lowy, 2008).

Bacterial plasmids analysis was the former molecular technique used for MRSA epidemiological investigation but has many limitations. The RAPD PCR (Random Amplified Polymorphic DNA PCR) also called as AP-PCR (Arbitrarily Primed PCR) is a variant of classic PCR (Neela *et al.,* 2005). This method also has the ability of identifying variation in genetic material and establishing specific strain fingerprints (Babalola, 2004). The basic aim of this research was to characterize MRSA strains collected from different patients so that spreading of MRSA can be controlled.

Materials and methods

Bacterial sample collection

A total of 23 samples were collected from patients attending tertiary human hospitals in Faisalabad city. The samples were collected from accidental and surgical wounds, burns, diabetic foot and human nares using sterilized cotton tip swabs. After collection the samples were set in cool box and transferred to laboratory for further processing.

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, Wilmingtion, 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.

RAPD (PCR) analysis

The RAPD primers custom synthesized from GENELINK (Catalag No. 40-0001-01. Hawthorne. NY) were used for RAPD (PCR). The total of 22 RAPD primers from A, K and L series were used for amplification. Sequences of the RAPD primers that showed amplification in the present study are provided in (Table-1).

RAPD-PCR reaction mixture consisted of 10X buffer solution [$(NH_4)_2SO_4$] Mg+ Free, 2.5mM Magnesium chloride ($MgCl_2$), dNTPs (0.1mM each), RAPD Primer (15ng), *Taq* DNA (Polymerase), Template DNA (10ng) and Ultra purified double distilled autoclaved water (d_3H_2O). Amplification was carried out in Thermal cycler (Applied Biosystems) under the following conditions: initial denaturation at 95 C for 10min, 40 cycles (denaturation at 95°C for 1min, 36°C for 30 s, and extension at 72°C for 1 min), and final extension at 72°C for 7min. Resolution of PCR products amplified by 29 SSR primers were visualized by 6% denaturing polyacrylamide gel (PAGE) followed by staining in ethidium bromide.

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

Genetic characterization of twenty three MRSA strains were analyzed using 22 RAPD primers of series A, L and K out of which 14 RAPD primers showed reliable banding pattern and 4 of the RAPD primers showed low amplification while 4 primers showed no amplification. Total of 827 RAPD fragments were amplified. Number of amplified fragments varied from 3 to 8. Primer GLL-2 and GLL-3 amplified highest number of bands (8) and primer GLA-1 amplified lowest number of bands i.e. 3. The level of polymorphism among MRSA isolates was 93.9% identified through RAPD markers (Table 1).

Table 1. Genetic polymorphism among 23 MRSA isolates.

Sr. No	Primer Name	Sequence (5′-3′)	TNB	TNL	NPB
1	GLL-01	GGCATGACCT	43	3	3
2	GLL-02	TGGGCGTCAA	92	8	6
3	GLL-03	CCAGCAGCTT	90	8	7
4	GLL-04	GACTGCACAC	45	3	3
5	GLL-05	ACGCAGGCAC	45	3	2
6	GLL-08	AGCAGGTGGA	99	7	6
7	GLA-01	CAGGCCCTT C	27	3	3
8	GLA-02	TGCCGAGCTG	67	5	5
9	GLA-03	AGTCAGCCAC	69	5	5
10	GLA-04	AATCGGGCTG	52	7	7
11	GLK-01	CATTCGAGCC	36	3	3
12	GLK-02	GTCTCCGCAA	39	3	3
13	GLK-03	CCAGCTTAGG	50	7	7
14	GLK-04	CCGCCCAAAC	73	6	6
Total			827	71	66

TNB= Total Number of Bands, TNL= Total Number of Loci, NPB= Number of Polymorphic Bands.

Genetic similarities and Relationship among MRSA isolates

Genetic similarity ranges from 88.73% to 52.11% (Table-2). Maximum genetic similarity was found between 143 and 136 while the minimum genetic similarity was found among 141 and 88. Genetic relationship between MRSA isolates was identified through cluster analysis by Unweighted Paired Group of Arithmetic Means Average (UPGMA) method. All 23 MRSA isolates were clustered into two main clusters A and B (Fig.1).

Cluster B contains one member 134 which showed 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.

Table 2. Genetic Similarity matrix of twenty three human MRSA isolates.

ID	138	139	141	1 43	144	122	123	126	133	134	136	137	145	146	95	97	98	35	40	75	88	16	17
138	****	0.8310	0.8451	0.7746	0.7606	0.6338	0.7042	0.6761	0.7746	0.7606	0.7746	0.7324	0.7324	0.7746	0.5775	0.6056	0.6197	0.6479	0.6338	0.6197	0.5634	0.5775	0.5775
139		****	0.8169	0.8310	0.7887	0.6620	0.7606	0.7887	0.7746	0.7887	0.7746	0.8451	0.7606	0.7465	0.6620	0.6056	0.7042	0.6197	0.6901	0.7042	0.6479	0.6620	0.6338
141			****	0.8169	0.8592	0.6197	0.7183	0.6901	0.7324	0.7183	0.7606	0.7183	0.8028	0.8169	0.5634	0.6197	0.6056	0.6056	0.5915	0.5775	0.5211	0.5634	0.5634
143				****	0.7887	0.5775	0.8451	0.8169	0.8028	0.7324	0.8873	0.7887	0.7606	0.8028	0.5775	0.5493	0.5915	0.5915	0.6338	0.5915	0.5634	0.5493	0.5493
144					****	0.5634	0.8028	0.6901	0.7606	0.6338	0.7324	0.7183	0.7465	0.7042	0.6197	0.6479	0.6338	0.6620	0.5915	0.6338	0.5493	0.5634	0.5634
122						****	0.5915	0.5915	0.5493	0.6479	0.6056	0.6479	0.6197	0.6056	0.6056	0.5211	0.6479	0.6761	0.6901	0.6479	0.5915	0.5493	0.5493
123							****	0.8028	0.7887	0.6901	0.7887	0.7746	0.7465	0.7606	0.5634	0.5634	0.6056	0.6901	0.6479	0.6056	0.6056	0.5915	0.5352
126								****	0.7606	0.7465	0.8451	0.8592	0.8028	0.7606	0.6197	0.5634	0.6901	0.6056	0.6197	0.6620	0.6620	0.6197	0.5915
133									****	0.7324	0.8028	0.7887	0.7606	0.7465	0.6338	0.6620	0.6761	0.6479	0.6620	0.6761	0.6197	0.6620	0.6056
134										****	0.7042	0.8592	0.7746	0.7324	0.6479	0.6197	0.7183	0.6338	0.7042	0.6901	0.6901	0.7324	0.6761
136											****	0.7887	0.7606	0.8028	0.5775	0.5493	0.5915	0.5915	0.5775	0.5634	0.5634	0.5211	0.5211
137												****	0.8310	0.7324	0.7042	0.6197	0.7183	0.6620	0.6761	0.7183	0.6901	0.7042	0.6761
145													****	0.8451	0.6479	0.6197	0.7183	0.6901	0.6197	0.6901	0.6056	0.5915	0.5352
146														****	0.5775	0.5493	0.6197	0.6761	0.6338	0.5915	0.5352	0.5493	0.4930
95															****	0.7746	0.7887	0.7324	0.7746	0.7606	0.8169	0.7746	0.7183
97																****	0.7324	0.6761	0.7465	0.7042	0.7324	0.7746	0.7183
98																	****	0.8028	0.7606	0.9155	0.8028	0.7606	0.7324
35																		****	0.8169	0.8592	0.7465	0.7324	0.5915
40																			****	0.8169	0.8169	0.8028	0.6901
75																				****	0.8028	0.7887	0.7042
88																					****	0.8732	0.7887
16																						****	0.8028
17																							****

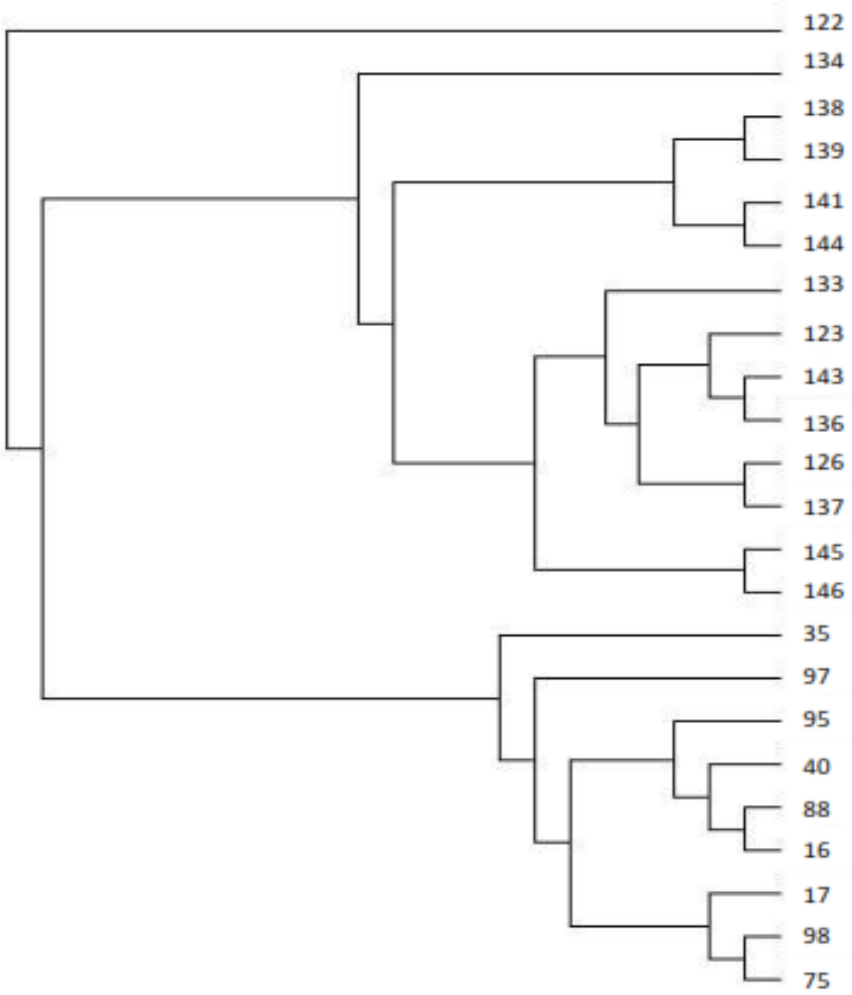

Fig. 1. Dendrogram of 23 MRSA isolates with RAPD data using Nei's genetic distances UPGMA method.

Discussion

Staphylococcus aureus bacterium is Gram-positive fit in to the Staphylococaceae family and is frequently originate as a commensal on the mucous membranes, skin and skin glands mainly in the nose of vigorous individuals (Plata *et al*., 2009).

Investigation of the epidemiology of *S. aureus* strains have been used a large variety of molecular methods that includes pulse-field gel electrophoresis, restriction fragment length polymorphism, multi locus sequence analysis and PCR based methods (Karakulska *et al*., 2011; Lee *et al*., 2011. In present study it was found that 10ng/µl gave the best results and it was finally used as an optimized DNA concentration for RAPD (PCR). Likewise 2.5mM dNTPs, 3mM $MgCl_2$, 3µl template DNA and 1U *Taq* DNA polymerase was found optimum for RAPD (PCR) in a total volume of 25µl reaction mixture.

In present study 14 out of tested 22 RAPD primers amplified reproducible and polymorphic PCR products and 71 RAPD loci were identified among twenty three MRSA samples with percentage of polymorphism were 93.9%. Genetic similarity among the MRSA isolates ranges from 88.73% to 52.11%. The maximum genetic similarity was found between 143 and 136 while the minimum genetic similarity was found among 141 and 88. Single RAPD primer that showed 45 major banding patterns from 108 MRSA isolates (Power, 1996). Neslihan Idil *et al* (2014) revealed 3 out of 10 RAPD primers yielded 96.9% maximum and 38.1% minimum genetic similarity. Manakant and Tanaya (2012) used 3 RAPD primers that revealed five different banding patterns (I-V) with 90% genetic similarity among 27 MRSA isolates. These results are quite comparable with the earlier findings of RAPD 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 RAPD analysis is present.

Acknowledgement

Authors acknowledge the department of Clinical Medicine and Surgery, University of Agriculture Faisalabad and Allied Hospital Faisalabad for providing the MRSA samples.

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