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Emergence Of New MLST Clones Of Staphylococcus *aureus* Bacteraemia In Paediatric Patients In North India

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Abstract

Introduction: Staphylococcus aureus bacteraemia is most common life threatening infection in paediatric population. Along with that methicillin resistance Staphylococcus aureus bacteraemia is associated with increased morbidity and mortality.

Multi-locus sequence typing sequences internal fragments of 7 housekeeping genes of Staphylococcus aureus from bacteraemia patients. This is highly discriminatory and can show that same allelic profile patients have same antibiotic sensitivity patterns. As MLST of Staphylococcus aureus is naïve for northern India, we planned to perform MLST of SAB for paediatric patients, so that allelic profiles of North Indian population can be generated.

Material and Methods – A total of 128 Staphylococcus aureus isolates from 2280 Paediatric patients were screened for MRSA by detecting presence of mecA, mecC genes. Out of these, 4 Isolates were processed for 7 MLST genes and Allelic profile was detected by sequencing and were submitted to PUBMLST software for ST and clonal complexes determination.

Result: Out of 128 isolates, 55 were mecA positive, 3 were mecC positive. 4 mecA isolates showed presence of 7 MLST genes. Allelic Profiles generated, 2 NICU patients had same Clonal Complex CC1, which is the most common clonal complex. Rest were CC22 (naïve isolate), CC-8(dangerous clonal type). CC1 patients had a good survival rate compared to others.

Conclusion: MLST should be done on all critical patients to know the disease outcome. MLST provides an unambiguous method of assigning MRSA isolates with clones to know strains of same origin and to detect the severity and disease outcome.

Keywords: Staphylococcus aureus bacteraemia, MLST, clonal typing, MRSA

Introduction

Staphylococcus aureus bacteraemia is one of the most common life-threatening infection in paediatric population.[1] The prevalence of Staphylococcus aureus bacteraemia ranges from 10-30/100000 population in the developing countries. This is due to lack of infection control practices and care from health care systems. Treatment with penicillin and its derivatives had led to a dramatic impact on mortality during the early 1900. But penicillinase producing Staphylococcus isolates had resistance to Penicillin G and penicillin V. These problems were solved by introduction of semi-synthetic penicillin methicillin. Further rampant use of methicillin led to MRSA which reported in Europe and USA initially in 1960-1970s.[2-3] These methicillin resistant Staphylococcus aureus strains were associated with increased morbidity and mortality. Many MRSA strains are less susceptible or resistant to glycopeptide antibiotics.[4] These MRSA strains are a major concern in hospitals and lead to difficulty in treating hospital acquired Staphylococcus aureus infection. [4]

The rising number of cases of SAB has led to detailed research regarding genetic background of the pathogen across different geographical regions. In recent times many modalities are available for typing and defining strains of Staphylococcus aureus.[5]These include PCR based methods, PFGE, MLST, WGS.

MLST characterises any bacterial isolate on the basis of sequencing of 450bp internal fragments of defined housekeeping genes. It is a DNA sequence-based typing method that is widely accepted.[5,6] This technique was initially developed for *Neisseria meningitidis*[7] ten subsequently used for assigning *Streptococcus pneumoniae* strains to the major hypervirulent clones[8,9] and major penicillin-resistant and multiple-antibiotic-resistant clones.[9]

For Staphylococcus aureus, MLST provides a wide level of discrimination which depicts the global dissemination of the organism.[5] This is highly discriminatory and can show that same allelic profile patients have same antibiotic sensitivity patterns. For each gene fragment, the different sequences are assigned as distinct alleles, and each isolate is defined by the alleles at each of the seven housekeeping loci (the allelic profile or sequence type [ST]). In addition, the data obtained by MLST can be used to address basic questions about the evolutionary and population biology of bacterial species.[6]

As MLST of Staphylococcus aureus was naïve for northern India, we planned to perform MLST for paediatric patients, so that allelic profiles of North India can be generated. In this report we validate MLST scheme from bacteraemia patients for *S. aureus* and demonstrate the utility of the method by identifying the MRSA clones in paediatric population.

Material and methods

Patient population

All suspected bacteraemia cases admitted in NICU, PICU and Paediatric wards at Department of Paediatrics, King Georges Medical university, were included in the study. The study period was 1 year (December 2018 to Nov 2019). Consent was obtained from parents / guardian of the patients.

Complete case history including risk factors was taken in a preformed questionnaire. This study was approved from institutional ethics committee.

Identification and anti-microbial sensitivity testing

Paired blood cultures were obtained from patients. Blood culture was performed on automated systems. Staphylococcus aureus was identified by colony characteristics along with biochemicals (Catalase, coagulase and mannitol salt agar). Repeat confirmation of strains was done by MALDI-TOF MS system. Antimicrobial sensitivity for cefoxitin and vancomycin, gentamycin, erythromycin, clindamycin, ciprofloxacin, doxycycline, linezolid, teicoplanin, trimethoprim-sulfamethoxazole, vancomycin, and daptomycin and cefoxitin was done according to CLSI guidelines.[10]

Detection of mecA, mecC genes for MRSA [11,12]Cefoxitin resistant MRSA strains were further evaluated for mecA, mecB and mecC. Detection was done using convention PCR with primers as specified in table 1

Table 1 -mecA and mecC Primer sequences for detection of MRSA strains

Genes	Primers	Primer sequence 5'-3'	PCR product length
mecA	Mec A-F mecA-R	AAA AAA GGT GGT ATC GAT TGG C AGT TCT GCA GTA CCG GAT TTG C	533bp
mecC	Mec C-F Mec C - R	GTCCCTAACAAAACACCCAAAGA GAAGATCTTTTCCGTTTTCAGC	454bp

Multi Locus Sequence Typing (MLST) of Staphylococcus aureus

This involves:

1. DNA extraction – This was done using 7 house keeping genes carbamate kinase (*arcC*), shikimate dehydrogenase (*aroE*), glycerol kinase (*glp*), guanylate kinase (*gmk*), phosphate acetyltransferase (*pta*), triosephosphate isomerase (*tpi*), and acetyl coenzyme A acetyltransferase (*yqiL*).[13] The DNA sequences of these genes are described in table2.
2. PCR (conventional)- PCR amplification was done by conventional PCR and products were amplified using agarose gel electrophoresis. As shown in fig 1.
3. Genome sequencing – it was outsourced.
4. Assembly of genes and assigning alleles- Assembly of genes was done using BLAST (http://www.ncbi.nlm.nih.gov/blast/blast.cgi). The sequence of the particular housekeeping genes from the *S. aureus* MLST scheme were manually extracted from the sequences and compared to references on the *S. aureus* MLST database (http://pubmlst.org/saureus).[14] Based on the sequence match classical sequence type (ST) was assigned.
5. Submission on MLST database for ST types - Novel sequences were submitted to the *S. aureus* MLST database http://pubmlst.org/saureus. The associated clonal complex (CC) was calculated using the eBURST algorithm (http://saureus.mlst.net/eBURST/), with CCs defined using a criterion of six common alleles.[14] Sequences have been deposited in the NCBI Sequence read archive under the authors name.

Table 2- primer Sequences used in PCR amplification

Gene	Primer	Sequence (5′-3′)	Length (bp)
Carbamate kinase (*arcC*)	*arcC*-Up	TTGATTCACCAGCGCGTATTGTC	456
	arcC-Dn	AGGTATCTGCTTCAATCAGCG	
Shikimate dehydrogenase (*aroE*)	*aroE*-Up	ATCGGAAATCCTATTTCACATTC	456
	aroE-Dn	GGTGTTGTATTAATAACGATATC	

Glycerol kinase (*glpF*)	*glpF*–Up	CTAGGAACTGCAATCTTAATCC	465
	glpF–Dn	TGGTAAAATCGCATGTCCAATTC	
Guanylate kinase (*gmk*)	*gmk*–Up	ATCGTTTTATCGGGACCATC	429
	gmk–Dn	TCATTAACTACAACGTAATCGTA	
Phosphate acetyltransferase (*pta*)	*pta*–Up	GTTAAAATCGTATTACCTGAAGG	474
	pta–Dn	GACCCTTTTGTTGAAAAGCTTAA	
Triosephosphate isomerase (*tpi*)	*tpi*–Up	TCGTTCATTCTGAACGTCGTGAA	402
	tpi–Dn	TTTGCACCTTCTAACAATTGTAC	
Acetyl coenzyme A acetyltransferase (*yqiL*)	*yqiL*–Up	CAGCATACAGGACACCTATTGGC	516
	yqiL–Dn	CGTTGAGGAATCGATACTGGAAC	

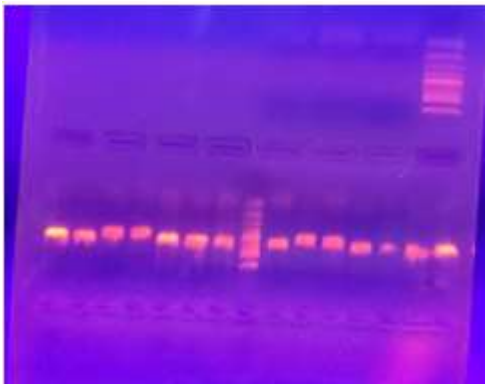

Fig 1: PCR amplification of 7 housekeeping genes on agarose gel electrophoresis

Results

From December 2018 to November 2019, 2280 patients were admitted with septicaemia in paediatric wards, NICU and PICU. Paired blood culture was obtained for detection of Staphylococcus aureus bacteraemia. SAB was identified in 128 (11.7%) patients which included 1085 positive blood cultures over the time of study. Distribution of patients based on age and sex has been shown in table 3.

Table no.3: Age and Sex wise distribution of Staphylococcus *aureus* bacteraemia isolates

	Number of Patients	Sex		Age		
		Male	Female	<1month	1month–1year	>1year
NICU	67	54	13	67	–	–
PICU	24	10	14	–	12	12
Paediatric ward	37	21	16	–	11	26

Out of 128 Staphylococcus *aureus* isolates, 61 patients were resistant to cefoxitin (MRSA) by disc diffusion and agar dilution methods. Amplification of the *mec*A gene was observed in 55 of the 61 MRSA isolates and mec C gene in 3 isolates. 3 MRSA isolates did not amplify mec A and mecC genes.

MLST typing revealed genetic diversity in Staphylococcus *aureus* bacteraemia isolates. In the subset of 128 SAB cases, we analysed clonal types of 4 mecA positive isolates admitted in NICU during the same time. It showed presence of all 7 MLST genes. Allelic profiles were generated, 2 NICU patients had same Clonal Complex CC1, which is the most common clonal complex. Rest were CC8 and CC22 (novel clonal type). CC1 patients had a good survival rate compared to others.

Table No 4: Genome testing and cloning pattern

Isolate	arcC	aroE	glpF	gmk	pta	tpi	yqiL	ST	clonal complex
B-604	1	1	1	1	1	41	286	6274	CC1
B-928	7	6	1	5	8	8	6	22	CC22
B-1104	543	3	1	1	4	4	3	6275	CC8
B-1289	1	1	1	1	1	41	286	6274	CC1

Discussion

In one of the biggest tertiary care centre in North India, this study revealed 128 cases of Staphylococcus aureus bacteraemia during the study period of 1 year.

Methicillin resistance was reported in nearly 47% cases which is very alarming & needs to be further evaluated. These strains pose major challenge in their treatment as methicillin is a protype for resistance to β- lactams & other agents like clindamycin & erythromycin. Detection of mec A, B, C is also important in guiding antimicrobial therapy. Mec A was isolated in 90% strain which show resistance to other group of antibiotics (erythromycin & clindamycin). Worldwide studies have demonstrated 57% prevalence of MRSA and mecA being outnumbered in MRSA strain.[15] mec A positive MRSA strain need to be checked for their clone types as there are associated with specific geographical locations.

Outbreaks of MRSA infections in paediatric population are described in studies and their clonal association with specific geographical locations has been reported world-wide. [16] MLST defined clones have shown relatedness to each other & to the MSSA & MRSA strains.

CC-1 (2 isolates), CC-8 & CC-22 were the clonal complexes defined in our study. CC-1 is dominant clone of CA – MRSA in USA.[18] In India, single study done in multi-centric setting published, suggests ST-97, ST-1 & ST-9 to be the prevalent clones. [19] cases of CC 8 and 22 have not been reported in India. This were first reported in our setting.

CC-1 has been shows to have good survival & response to the antimicrobial therapy.[19,20] CC-8 has been reported as a clone with global dissemination having poor out come in children. CC8- MRSA bacteremia strain is associated with high mortality dur to antimicrobial resistance. In our study also CC-1 strain patients responded to the antimicrobial therapy & had a good outcome. The patients with strain CC8 were not see to survive irrespective of the antimicrobial therapy. A study done of paediatric population on SAB, reports CC8 strain which is MRSA was associated with increased mortality due to resistance with first line of empirical treatment. [21]

North India scenario of MLST of *Staphylococcus aureus* need to be further evaluated with a wide range of strains undergoing clonal typing. This will help in understanding the transmission pattern & the effectiveness of antimicrobial therapy. This was a nine-study done to standardize MLST in our setting which led to identifying new clone CC-22 as a potential cause of SAB in pediatric & neonatal population.

MLST though a tedious but a remarkable method of assigning clonal types to staphylococcus aureus. Once standardized, it can be done on large population in no time. This will help in understanding the clones of *Staphylococcus aureus* & their various clonal types circulating in the population.

Conclusion

MLST should be done on all critical patients to know the disease outcome. MLST provides an unambiguous method of assigning MRSA isolates with clones to know strains of same origin.

As of now this is the first study done in Northern India on MLST staphylococcus aureus. And irrespective of limited resources we were successfully able to identify 3 clones (2 common and 1 naïve) on Staphylococcus aureus circulating in our geographical region.

References

1. Miko BA, Hafer CA, Lee CJ, Sullivan SB, Hackel MA, Johnson BM, et al. Molecular characterization of methicillin-susceptible Staphylococcus aureus clinical isolates in the United States, 2004 to 2010. J Clin Microbiol. 2013; 51(3):874–9. Epub 2013/01/04. https://doi.org/10.1128/JCM.00923-12 PMID: 23284029
2. Panlilio A L, Culver D H, Gaynes R P, Bannerjee S, Henderson T S, Tolson J S, Martone W J. Methicillin-resistant Staphylococcus aureus in US hospitals, 1975–1991. Infect Control Hosp Epidemiol. 1992;13:582–586. [PubMed] [Google Scholar] [Ref list]
3. Speller D C, Johnson A P, James D, Marples R R, Charlett A, George R C. Resistance to methicillin and other antibiotics in isolates of Staphylococcus aureus from blood and cerebrospinal fluid, England and Wales, 1989–1995. Lancet. 1997;250:323–325. [PubMed] [Google Scholar] [Ref list]
4. Kim ES, Kim HB, Kim G, Kim KH, Park KH, Lee S, et al. Clinical and epidemiological factors associated with methicillin resistance in community-onset invasive Staphylococcus aureus infections: prospective multicenter cross-sectional study in Korea. PLoS One. 2014; 9(12):e114127. Epub 2014/12/09. https:// doi.org/10.1371/journal.pone.0114127 PMID: 25485895
5. Maiden M C J, Bygraves J A, Feil E, Morelli G, Russell J E, Urwin R, Zhang Q, Zhou J, Zurth K, Caugant D A, Feavers I M, Achtman M, Spratt B G. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc Natl Acad Sci USA. 1998;95:3140–3145. [PMC free article] [PubMed] [Google Scholar]
6. Spratt B G. Multilocus sequence typing: molecular typing of bacterial pathogens in an era of rapid DNA sequencing and the Internet. Curr Opin Microbiol. 1999;2:312–316. [PubMed] [Google Scholar]
7. Feil E J, Maiden M C J, Achtman M, Spratt B G. The relative contributions of recombination and mutation to the divergence of clones of Neisseria meningitidis. Mol Biol Evol. 1999;16:1496–1502. [PubMed] [Google Scholar]
8. Enright M, Spratt B G. A multilocus sequence typing scheme for Streptococcus pneumoniae: identification of clones associated with serious invasive disease. Microbiology. 1998;144:3049–3060. [PubMed] [Google Scholar]
9. Enright M C, Fenoll A, Griffiths D, Spratt B G. The three major Spanish clones of penicillin-resistant Streptococcus pneumoniae are the most common clones recovered in recent cases of meningitis in Spain. J Clin Microbiol. 1999;37:3210–3216. [PMC free article] [PubMed] [Google Scholar]
10. M100, National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing. Ninth informational supplement. Villanova, Pa: National Committee for Clinical Laboratory Standards; 2018. [Google Scholar]
11. Kumurya AS, Gwarzo MY, and Uba A (2015) One Step PCR for Detection of Staphylococcus aureus specific Sequence gene and mecA gene. International Journal of Advanced Materials Research. 1(3):73-79.

12. Pournajat A, Ardebili A, Goudarzi L, Khodabandeh M, Narimani, and T Abbaszadeh H (2014) PCR based identification of Methicillin-resistant Staphylococcus aureus strains and their antibiotic resistance profiles. Asian Pacific Journal of Tropical Biomedicine. 4: S293-S297.
13. Saunders NA, Holmes A. Multilocus sequence typing (MLST) of Staphylococcus aureus. Methods Mol Biol. 2007;391:71-85. doi: 10.1007/978-1-59745-468-1_6. PMID: 18025670.
14. Feil EJ, Li BC, Aanensen DM, Hanage WP, Spratt BG. eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. J Bacteriol. 2004;186(5):1518-30. pmid:14973027
15. Abdulgader SM, Shittu AO, Nicol MP, Kaba M. Molecular epidemiology of Methicillin-resistant Staphylococcus aureus in Africa: a systematic review. Front Microbiol. 2015 Apr 30;6:348. doi: 10.3389/fmicb.2015.00348. PMID: 25983721; PMCID: PMC4415431.
16. Chuang YY, Huang YC. Molecular epidemiology of community-associated meticillin-resistant Staphylococcus aureus in Asia. Lancet Infect Dis. 2013;13(8):698-708. pmid:23827369
17. Koser CU, Holden MT, Ellington MJ, Cartwright EJ, Brown NM, Ogilvy-Stuart AL, et al. Rapid whole-genome sequencing for investigation of a neonatal MRSA outbreak. The New England journal of medicine. 2012;366(24):2267-75. Epub 2012/06/15. pmid:22693998
18. MS Aung, N Urushibara, M Kawaguchiya, M Hirose, M Ito, S Habadera, et al. Clonal diversity of methicillin-resistant Staphylococcus aureus (MRSA) from bloodstream infections in northern Japan: identification of spermidine N-acetyltransferase gene (speG) in staphylococcal cassette chromosomes (SCCs) associated with type II and IV SCCmecJ Glob Antimicrob Resist, 24 (2021), pp. 207-214, 10.1016/j.jgar.2020.12.008
19. Brahma U, Suresh A, Murthy S, Bhandari V, Sharma P. Antibiotic Resistance and Molecular Profiling of the Clinical Isolates of Staphylococcus aureus Causing Bovine Mastitis from India. Microorganisms. 2022 Apr 18;10(4):833. doi: 10.3390/microorganisms10040833. PMID: 35456882; PMCID: PMC9024461.
20. Recker, M., Laabei, M., Toleman, M. S., Reuter, S., Saunderson, R. B., Blane, B., et al. (2017). Clonal differences in Staphylococcus aureus bacteraemia-associated mortality. Nat. Microbiol. 2, 1381-1388. doi: 10.1038/s41564-017-0001-x
21. Garrine, M., Quintó, L., Costa, S. S., Messa, A., Massinga, A. J., Vubil, D., et al. (2023). Epidemiology and clinical presentation of community-acquired Staphylococcus aureus bacteraemia in children under 5 years of age admitted to the Manhiça District hospital, Mozambique, 2001-2019. Eur. J. Clin. Microbiol. Infect. Dis. 42, 653-659. doi: 10.1007/s10096-023-04580-2