Understanding the Local Epidemiology of Methicillin-Resistant Staphylococcus aureus by Pulsed-Field Gel Electrophoresis in an Indian Tertiary Care Centre

Avinandan Saha (saha.avinandan@gmail.com)
Seth GS Medical College and KEM Hospital: King Edward Memorial Hospital and Seth Gordhandas Sunderdas Medical College

Priyanka Prasad
Seth GS Medical College and KEM Hospital: King Edward Memorial Hospital and Seth Gordhandas Sunderdas Medical College

Gita Nataraj
Seth GS Medical College and KEM Hospital: King Edward Memorial Hospital and Seth Gordhandas Sunderdas Medical College

Research

Keywords: Methicillin-resistant Staphylococcus aureus, pulsed-field gel electrophoresis, molecular epidemiology, hospital-acquired infections, horizontal transmission, infection prevention and control

DOI: https://doi.org/10.21203/rs.3.rs-281771/v1

Abstract

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of community and hospital-acquired infections (HAIs). In the year preceding this study, our laboratory reported an MRSA isolation rate of 2% from 50,549 specimens. Molecular typing of MRSA identifies sources of infection, transmission chains and informs infection control practices, and pulsed-field gel electrophoresis (PFGE) is the gold standard. This study was conducted to gain an understanding of the local molecular epidemiology of MRSA in our hospital using PFGE, to inform hospital infection control practices.

Methods: This prospective longitudinal study was conducted in the microbiology laboratory of our 2,200-bed tertiary care teaching hospital in Mumbai, India.

The antibiotic susceptibility profiles and pulsed-field profiles (PFPs) of 100 consecutive non-duplicate clinical isolates of MRSA were obtained. The PFPs were compared to check for relatedness of isolates. The distribution of various pulsotypes across disciplines and hospital locations was examined.

Results: Clinical specimens accounted for 86 (86%) of the MRSA isolates, whilst 14 (14%) were from screening of healthcare workers. Maximum isolates, 68 (68%), were from surgical disciplines. Confirmed HAIs accounted for 25 (25%) MRSA isolates. Seventeen antibiotypes were obtained and there was no correlation between antibiotype and pulsotype. Totally 43 pulsotypes were identified, with most isolates, 40 (40%), belonging to pulsotype 1. Seven clusters were identified. Cluster I had maximum pulsotypes, 14, and 58 (58%) isolates. Isolates belonging to clusters I and II were found in all hospital locations. Relatedness was observed between isolates from HAIs and screening specimens, and between community and HAI isolates.

Conclusions: PFGE typing revealed the disciplines at greatest risk from MRSA in our hospital. The commonality between MRSA isolated from HAIs and screening of healthcare workers, and between MRSA isolated from HAIs and from community-acquired infections highlighted the horizontal transmission of MRSA and the need to reinforce infection control measures to limit this.

Background

Methicillin-resistant *Staphylococcus aureus* (MRSA) causes illnesses that are difficult to treat as they are resistant to β-lactam antibiotics and, frequently, multiple other antimicrobials [1,2]. Selection pressure arising from the inappropriate use of antibiotics has led to MRSA becoming a concern in healthcare settings and in the community [1,3]. Its propensity to colonise human skin, form surface biofilms, and transmissibility through direct skin contact or via fomites make MRSA an important cause of hospital-acquired infections (HAIs) [1,2,4,5].

Typing of MRSA is central to controlling its spread, enabling early detection of outbreaks, identifying sources of infection, transmission chains and informing infection control practices [6,7]. Pulsed-field gel electrophoresis (PFGE) is a band-based genotyping method. The chromosomal DNA of MRSA is digested

with the rare-cutting restriction enzyme *Sma*I derived from *Serratia marcescens*. This enzyme cleaves specifically at CCC^GGG sites on the chromosome, yielding DNA fragments that range in size from 10 kilobases – 800 kb [8]. The digested DNA fragments are separated by agarose gel electrophoresis in an electric field with an alternating voltage gradient. The resultant banding patterns produced by different isolates are compared to determine their relatedness [6,8]. The advantages of PFGE that make it the gold standard for epidemiological typing of MRSA are: its high discriminatory index (> 0.95), concordance of greater than 95% with sequence-based genotypic methods, viz., multilocus sequence typing (MLST) and Staphylococcal protein A (*spa*) typing, and lower cost [6,9].

India is a high prevalence country for MRSA, with a prevalence of 37% (21%-45%) [10]. It is estimated that 55%-65% of *S. aureus* isolates from India are resistant to cefoxitin [11]. Data on MRSA carrier rates vary widely from 1.8% among healthy community-dwelling population to 23% among healthcare workers (HCWs). The epidemiology and transmission of MRSA in Indian healthcare settings has only been sparsely studied at a molecular level [12-15].

The aim of this study was to characterise MRSA from clinical isolates in our centre by PFGE profiles (pulsotypes), examine the relatedness of various isolates, and determine the distribution of MRSA pulsotypes across hospital locations. This was done to understand the local epidemiology of MRSA at our centre at the molecular level. This understanding would inform the development and implementation of hospital infection control policies tailored to our unique situation.

Methods

Study setting, design and duration:

This prospective longitudinal study was carried out in the microbiology laboratory of a 2,200-bed tertiary care teaching hospital in Mumbai, India over 18 months. In the year preceding this study, our laboratory reported an MRSA isolation rate of 2% from 50,549 specimens processed for bacteriology. Disciplines from which MRSA was predominantly isolated (72% of all MRSA) were general surgery, paediatric surgery, burns unit and orthopaedics. Our department also periodically screens HCWs for MRSA carriage.

Definitions of terms used:

Isolate: Pure culture of MRSA derived from a single colony of a single organism [8].

Pulsed-field profile (PFP): Banding pattern resolved by an MRSA isolate on PFGE [8].

Pulsotype: Isolates showing identical banding patterns [8,16].

Cluster: A group of isolates that produced PFGE banding patterns that were more than 80% similar, and that could be distinguished from other isolates on this basis [8,17,18].

Singleton: Isolate with a unique PFP not belonging to any of the identified clusters [17,18].

Sample size, inclusion and exclusion criteria:

One hundred (100) consecutive non-duplicate isolates of MRSA from specimens received in the microbiology laboratory during the study period were included. Isolates of MRSA obtained from HCWs who underwent routine screening as part of hospital infection control protocol during the study period were also included. Screening was not carried out exclusively for the purpose of this study.

Repeated MRSA isolates: (i) from the same site from the same patient isolated during the same course of hospitalisation and, (ii) in case of outpatients, within two weeks of previous isolation in our laboratory, were excluded.

All specimens were processed according to standard protocols for isolation, identification and antimicrobial susceptibility testing of MRSA [19,20].

Typing of MRSA isolates by PFGE:

The Centres for Disease Control's PulseNet protocol was followed and is briefly described below [21].

Four or five colonies of the test isolate were inoculated into 5 ml brain heart infusion broth (HiMedia, India), vortexed and incubated at 35°C for 24 h. After incubation, the concentration of each cell suspension was adjusted with sterile normal saline to an absorbance of 0.9 to 1.1 at 610 nm using a spectrophotometer (JLab, India).

Two hundred microlitres of the adjusted cell suspensions were centrifuged at 10,000 rpm for six minutes. The pellets were resuspended in 300 µl of Tris-EDTA (TE) buffer (ThermoFisher, USA) and equilibrated in a 37°C dry bath. Cell lysis was carried out by adding 4 µl conventional lysostaphin solution (Sigma-Aldrich, USA) to each tube. The tubes were incubated in a 37°C dry bath for an hour till the solutions had cleared.

After incubation, 300 µl of 1.8% w/v PFGE grade agarose (Sisco Research Laboratories, India) equilibrated to 55°C was added to the cell suspensions in each tube, mixed gently and 100 µl each dispensed into the wells of a small disposable plug mould (Bio-Rad, USA). The plugs were solidified at room temperature and incubated in EC lysis buffer overnight at 37°C.

The plugs were then washed in 4 ml TE buffer by agitating the tubes on an orbital rocker for 30 min after decanting the EC lysis buffer. The wash cycle was repeated four more times, using fresh TE buffer each time. The plugs were stored in TE buffer at 2°C - 8°C till use.

The plugs were removed from TE buffer and each cut to a size of 5 mm x 5 mm using a sterile blade to fit a 15-tooth comb. These were equilibrated at room temperature for 30 min in 200 µl of a 1:10 mixture of 10X CutSmart restriction buffer (New England BioLabs, USA) and Type I water. Following this, all the buffer-water mixture was aspirated from each tube and the plugs incubated overnight at 25°C in a 200 µl

mixture of *Sma*I restriction enzyme (New England BioLabs, USA) in 1:10 10X CutSmart restriction buffer and Type I water.

A 1% w/v PFGE grade agarose gel was prepared in 0.5X Tris-borate-EDTA (TBE) buffer (ThermoFisher, USA). Twelve plugs were included in each PFGE run along with three lambda reference standards [22]. Plug slices were loaded at the ends of the comb teeth. Reference standards of Lambda PFG Ladder (New England BioLabs, USA) of 1 mm thickness were sliced from the gel syringe and loaded in the first, middle (8^{th}) and last (15^{th}) teeth of the comb. Agarose equilibrated to 55°C was poured into the gel casting platform and allowed to solidify at room temperature for one hour.

A contour-clamped homogeneous electric field (CHEF) apparatus, the Bio-Rad CHEF Mapper system (Bio-Rad, USA), was used. Running parameters were: running buffer – 0.5X TBE, flow rate = 1 l/min, temperature = 14°C, volts = 200 V (6 V/cm), initial switch time = 5 s, final switch time = 40 s, time = 21 h, ramping – linear.

After completion of the run, the gel was stained in a 1.5 g/ml ethidium bromide solution (HiMedia, India) for 20 min in a covered container and de-stained in fresh distilled water for 45 min. The gel was documented using a Syngene gel doc system (Synoptics, UK) and the image saved in TIFF format with a minimum resolution of 768 x 640 pixels for further analysis [21,23].

Data analysis:

Antimicrobial susceptibility patterns were analysed for the phenotypic relatedness of isolates. Isolates with susceptibility differing for two or fewer drugs of the same class (not including cefoxitin) were classified in a single antibiotype.

Pulsed-field profiles of the isolates were compared by visual assessment based on the criteria (Table 1) proposed by Tenover et al [8]. These criteria were applied only if PFGE resolved at least 10 distinct bands per isolate. In case fewer than 10 bands were resolved on two repeat runs, or smearing was observed on two repeat runs (one with 0.5% SDS added prior to loading to free up the DNA from restriction enzyme), the isolate was classified as untypeable by PFGE [24].

A dendrogram was created to identify percent similarities. This was derived from the unweighted pair group method using arithmetic averages and Dice coefficients [23]. The various MRSA pulsotypes were mapped across hospital locations.

Results

The 100 non-duplicate consecutive MRSA isolates studied were obtained from 52 (52%) male and 48 (48%) female subjects. The median age of subjects from which MRSA was isolated was 28 y (range 1 d – 65 y).

Of the 100 MRSA isolates, 14 (14%) were obtained from HCWs from whom it was isolated during screening. Eleven were from clinical staff of obstetrics and gynaecology and three from general surgery. MRSA was not isolated from screening of staff of other disciplines carried out during the study period. The remaining 86 (86%) isolates were obtained from clinical specimens. The disciplines from which MRSA were isolated were: general surgery (21 isolates), obstetrics and gynaecology (18), orthopaedics (15), paediatric surgery (14), burns unit (9), otorhinolaryngology (6), dermatology (5), paediatrics (4), urology (2), internal medicine (2), and one each from haematology, gastrointestinal surgery, cardiothoracic surgery and neonatology.

Confirmed HAIs accounted for 25 (25%) of the MRSA isolates in this study. Of these, 22 (22%) were from surgical site infections (SSIs), two (2%) from central line-associated bloodstream infections (CLABSIs) and one (1%) from catheter-associated urinary tract infection (CAUTI).

The antimicrobial susceptibility patterns of the isolates are shown in Figure 1. The 100 MRSA isolates were assigned to 17 antibiotypes (Table 2). Antibiotypes Ab1, Ab2, Ab6, Ab11 and Ab12 had subtypes that included isolates with inducible clindamycin resistance. Antibiotypes Ab8 and Ab9 had subtypes that included isolates with intermediate susceptibility to erythromycin. Antibiotype Ab4 had a subtype comprising a single MRSA isolate with intermediate susceptibility to vancomycin (minimum inhibitory concentration 4 mg/l).

On PFGE, a total of 43 PFPs were resolved by 99 of the 100 MRSA isolates (Fig. 2). One isolate was untypeable by PFGE. Maximum number of isolates, 40 (40%) from across disciplines and hospital locations belonged to pulsotype 1 (Fig. 3). Seven clusters were identified (Figs. 2, 3, 4). Cluster I comprised 14 pulsotypes and the maximum number of isolates, i.e., 58 (58%). There were 16 (16%) singletons (Fig. 3).

The relationship between isolates obtained from HAIs and screening from various disciplines is shown in Figure 4.

In cluster I, 29 MRSA isolates from community-acquired infections were closely related to HAI and screening isolates. Similar observations were made from clusters II (six isolates from community-acquired infections), IV (one isolate) and VII (one isolate) (Fig. 3).

The clinical departments in our hospital are distributed between four buildings within an area of approximately 10 acres. Building A houses the departments of dermatology, internal medicine, general surgery, obstetrics and gynaecology, the burns unit, paediatrics, and paediatric surgery. Building B houses neonatology, gastroenterology and gastrointestinal surgery, neurology, neurosurgery, haematology and the laboratories. Building C houses orthopaedics, and Building D the cardiology, cardiothoracic surgery and pulmonology departments. The distribution of the various clusters between hospital buildings is shown in Figure 5 (screening isolates were not included in this figure as these were considered to be dispersed throughout the hospital).

Discussion

Of the 100 MRSA isolates studied, 87% were from the surgical disciplines of general surgery, orthopaedics, paediatric surgery and obstetrics and gynaecology. Skin and soft tissue infections (SSTIs) were the commonest presentation (48%). Maximum isolates (76%) were from wound swabs and pus specimens. Community infections accounted for 47% of our isolates. *S. aureus* is known to be the leading cause of SSTIs [10,25]. Since a majority of our isolates were from SSTIs for which therapy required surgical intervention, most of our isolates were obtained from specimens submitted by the surgical disciplines.

HAIs accounted for 25% of our isolates, with SSIs being the commonest (22%), followed by CLABSI (2%) and CAUTI (1%). MRSA is isolated more frequently from inpatients than from the community, and SSTIs are the commonest source [26,27].

The antimicrobial susceptibility patterns of MRSA isolates in our study agree with current trends in India and globally [10,26]. However, resistance to aminoglycosides, tetracycline, clindamycin and trimethoprim-sulfamethoxazole was infrequently encountered. The latter three are not commonly used in our hospital. Interestingly, although aminoglycosides are frequently used, resistance was infrequent.

Maximum isolates belonged to antibiotype Ab2 (Table 2), and were obtained from all hospital locations. Expectedly, there was no correlation between antibiotype and pulsotype as antibiotic susceptibility patterns depend on antibiotic usage [16,28]. For example, antibiotype Ab2 had isolates belonging to such diverse clusters I, II, IV, V, VI, as well as several singletons (Fig. 3).

The first report from India on the molecular typing of MRSA using PFGE was by Gayatri, et al. in 2005 [14]. Genotyping of 82 single-patient isolates of MRSA from two major hospitals revealed four major types and 24 subtypes. Also, isolates that belonged to the same strain by MLST and *spa* typing were found to be diverse by PFGE. Relatedness was observed to the Hungarian strain HUSA304 and the Brazilian strain HSJ216 that were circulating at the time [14]. In 2010, PFGE established the presence of EMRSA-15 variants in India in hospital and community in urban and rural settings, and grouped all pulsotypes into a single cluster as they were more than 80% similar [15]. We did not include any known MRSA strains in our PFGE experiments as the primary aim of our study was to understand the local epidemiology of MRSA in our hospital in terms of sources and transmission.

In our study, 99 MRSA isolates resolved into 43 pulsotypes belonging to seven clusters and there were 16 singletons. Cluster I was predominant with the maximum number of pulsotypes (14) and isolates (58%) (Fig. 3). Similar studies examining the molecular epidemiology of MRSA in hospital settings also reveal considerable molecular diversity of circulating MRSA but nearly always a single strain predominates. Jain, et al. typed 46 MRSA isolates from infected orthopaedic implants, grouping them into nine clusters based on a similarity cut-off of 80%, with one cluster being predominant. In their study, maximum isolates from across PFGE clusters were found by MLST to belong to the same clonal complex, indicating considerable diversity within members of the same complex [17]. Fomda, et al. assigned 154 MRSA

isolates from their tertiary care centre into ten pulsotypes, suggesting less diversity than that found in our centre. However, the origins of their isolates with regard to hospital or community are not elucidated [29]. Krishnan, et al. also assigned 65 MRSA isolates from burns wounds to 12 pulsotypes, with one pulsotype predominating [30].

We isolated MRSA belonging to cluster I from all four of our hospital buildings (Fig. 5). This was followed by isolates belonging to cluster II which were found in three of four buildings. Building A, the design and layout for which date back to 1926, houses the general clinical disciplines with approximately 900 beds in total, and is the most crowded. This building accounted for both maximum number and diversity of MRSA. This was followed by Building C, which houses orthopaedics and has 200 beds. Orthopaedics accounted for a considerable number of MRSA isolates (15%) as well as diversity in our study. Buildings B and D, which have a modern layout and design, house the super-specialty departments and are considerably less crowded, accounted for less number and diversity of MRSA (Fig. 5).

Carriage of MRSA by HCWs is a proven risk factor for HAIs [28,31]. In this study, isolates obtained from HAIs and screening specimens were, for the most part, related (Fig. 4). For example, cluster I comprised isolates from three SSIs from obstetrics and gynaecology, and general surgery, as well as nine screening isolates form the same disciplines. Interestingly, we isolated an MRSA belonging to cluster I from an infected copper-T that had been inserted several years ago in another hospital in the same city indicating possible widespread circulation of this strain in our city.

As many as 20% of HCWs in a hospital may carry MRSA at any point in time [31,32]. The genetic relatedness of screening isolates and isolates from HAIs in our study (Fig. 4) illustrates how failure to comply with infection control practices results in the transmission of MRSA between HCWs and patients. Dar, et al. also showed by PFGE the genetic relatedness of MRSA isolated from orthopaedic SSIs and screening specimens [28].

In clusters I, II, IV and VII, MRSA isolates from community-acquired infections were found to be closely related to isolates from HAIs (Fig. 3). Attempts at controlling MRSA within a hospital setting have been known to be hampered by the repeated influx of MRSA strains from the community [33]. Preventing this influx and also preventing the efflux of MRSA from the hospital into the community demands stringent adherence to infection control practices as well as antimicrobial stewardship to prevent the emergence of resistance in the first place [34]. Screening inpatients on admission and decolonisation of carriers has been shown to be successful in controlling the spread of MRSA [35,36].

A single isolate in our study was deemed untypeable by PFGE as band separation could not be achieved. A possible explanation could be that cleavage by the restriction endonuclease was blocked by methylation of the bacterial DNA. Members of MRSA ST398 are untypeable by *Sma*I PFGE due to the presence of a novel DNA methyltransferase [37].

Our study has a few limitations. Screening HCWs for MRSA was not carried out for the purpose of this study. Only MRSA isolates obtained from screening of disciplines carried out routinely as part of hospital

infection control protocol during the study period were included. Environmental sampling was not carried out. It is therefore possible that some sources of MRSA may have been missed. Secondly, inter-laboratory comparisons and identification of our isolates with known epidemic strains of MRSA could not be carried out as these were not included in our PFGE runs [6]. Sequence-based typing by SCC*mec*, MLST and *spa* would yield this information and place our findings in the broader context of national and global epidemiology of MRSA, providing a complete macro-epidemiological picture.

Nevertheless, this was the first time a study of the molecular epidemiology of MRSA in our centre was undertaken. This study highlighted the disciplines at greatest risk from MRSA transmission, viz., the surgical disciplines. Intensifying surveillance and control measures on these disciplines would limit transmission. The study revealed the commonality between MRSA isolated from HAIs and screening specimens, impressing the need to reinforce basic infection control measures, such as hand hygiene and audits, in areas at maximum risk. The commonality observed between MRSA isolated from HAIs and those from community-acquired infections suggested the need to screen admitted patients for MRSA carriage and to decolonise carriers (at least in areas at greatest risk), as well as reinforce basic infection control measures and antimicrobial stewardship. Finally, a protocol for the gold-standard PFGE typing was standardised and successfully employed that could also be used in future epidemiological investigations.

This study of the molecular epidemiology of MRSA by PFGE in our hospital thus served as an important tool for informing infection control practices and enabling targeted prevention of HAIs with economical utilisation of resources.

Conclusions

Typing by PFGE revealed the genetic diversity of MRSA in our centre. Isolates belonging predominantly to two clusters were found circulating throughout our hospital. Isolates from HAIs and screening specimens were related, suggesting horizontal transmission between HCWs and patients. Commonality was also observed between hospital and community isolates, suggesting possible introduction into the hospital from the community or vice versa.

Abbreviations

CAUTI: Catheter-associated urinary tract infections

CHEF: Contour-clamped homogeneous electric field

CLABSI: Central line-associated bloodstream infections

HAI: Hospital-acquired infections

HCW: Healthcare workers

MLST: Multilocus sequence typing

MRSA: Methicillin-resistant *Staphylococcus aureus*

PFGE: Pulsed-field gel electrophoresis

PFP: Pulsed-field profiles

spa: Staphylococcal protein A

SSI: Surgical site infections

SSTI: Skin and soft tissue infections

TBE: Tris-borate-EDTA

TE: Tris-EDTA

Declarations

Availability of data and materials

The datasets used and/or analysed during the present study are available from the corresponding author on reasonable request.

Acknowledgements

We would like to thank Dr Smita Mahale and Dr Vikrant Bhor of the National Institute of Research in Reproductive Health, Mumbai, India for kindly permitting use of their PFGE apparatus.

Funding

This study was supported by a grant from the Research Society of the Seth Gordhandas Sunderdas Medical College and King Edward VII Memorial Hospital, Mumbai, India.

Author information

Affiliation

Department of Clinical Microbiology, Seth Gordhandas Sunderdas Medical College and King Edward VII Memorial Hospital, Mumbai, India.

Avinandan Saha, Priyanka Prasad, Gita Nataraj.

Contributions

AS, PP, GN conceptualised the study. AS and PP collected and identified MRSA isolates, and performed antimicrobial susceptibility testing. AS performed PFGE of the MRSA isolates, the data analysis, and prepared the first draft of the manuscript. PP and GN reviewed, provided critical feedback and contributed to subsequent draft. All authors read and approved the final manuscript.

Corresponding author

Correspondence to Gita Nataraj. E-mail: gitanataraj@gmail.com

Ethics declarations

Ethics approval and consent to participate

Institutional ethics committee approval was obtained prior to commencing this study. As this was a laboratory-based study carried out on bacterial isolates and all patient data was anonymised, the institutional ethics committee waived the requirement for a consent to participate.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Lakhundi S, Zhang K. Methicillin-resistant *Staphylococcus aureus*: molecular characterization, evolution, and epidemiology. Clin Microbiol Rev [Internet]. 2018;31:e00020-18. Available from: http://cmr.asm.org/content/31/4/e00020-18.abstract.
2. Turner NA, Sharma-Kuinkel BK, Maskarinec SA, et al. Methicillin-resistant *Staphylococcus aureus*: an overview of basic and clinical research. Nat Rev Microbiol 2019;17:203–18.
3. Jarvis WR, Jarvis AA, Chinn RY. National prevalence of methicillin-resistant *Staphylococcus aureus* in inpatients at United States health care facilities, 2010. Am J Infect Control 2012;40:194–200.
4. Stevens AM, Hennessy T, Baggett HC, Bruden D, Parks D, Klejka J. Methicillin-resistant *Staphylococcus aureus* carriage and risk factors for skin infections, Southwestern Alaska, USA. Emerg Infect Dis 2010;16:797–803.
5. Mollema FPN, Richardus JH, Behrendt M, et al. Transmission of methicillin-resistant *Staphylococcus aureus* to household contacts. J Clin Microbiol 2010;48:202–7.
6. Deurenberg RH, Stobberingh EE. The evolution of *Staphylococcus aureus*. Infect Genet Evol. 2008;8:747–63.

7. Rebic V, Budimir A, Aljicevic M, Bektas S, Vranic SM, Rebic D. Typing of methicillin resistant *Staphylococcus aureus* using DNA fingerprints by pulsed-field gel electrophoresis. Acta Inform Medica 2016;24:248–52.
8. Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed- field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol. 1995;33:2233–9.
9. Hallin M, Deplano A, Denis O, De Mendonça R, De Ryck R, Struelens MJ. Validation of pulsed-field gel electrophoresis and spa typing for long-term, nationwide epidemiological surveillance studies of *Staphylococcus aureus* J Clin Microbiol 2007;45:127–33.
10. Rajkumar S, Sistla S, Manoharan M, et al. Prevalence and genetic mechanisms of antimicrobial resistance in Staphylococcus species: A multicentre report of the Indian Council of Medical Research Antimicrobial Resistance Surveillance Network. Indian J Med Microbiol 2017;35:53–60.
11. Global Antimicrobial Resistance Surveillance System (GLASS) Report. Geneva, World Health Organization. WHO. 2017.
12. Nithya V, Rathinam S, Karthikeyan SGR, Lalitha P. A ten year study of prevalence, antimicrobial susceptibility pattern, and genotypic characterization of methicillin resistant *Staphylococcus aureus* causing ocular infections in a tertiary eye care hospital in South India. Infect Genet Evol 2019;69:203–10.
13. Patel A, Patel K, Patel K, Shah S, Dileep P. Time trends in the epidemiology of microbial infections at a tertiary care center in west India over last 5 years. J Assoc Physicians India. 2010;58:S37–40.
14. Arakere G, Nadig S, Swedberg G, Macaden R, Amarnath SK, Raghunath D. Genotyping of methicillin-resistant *Staphylococcus aureus* strains from two hospitals in Bangalore, South India. J Clin Microbiol 2005;43:3198-3202.
15. Nadig S, Ramachandra Raju S, Arakere G. Epidemic meticillin-resistant *Staphylococcus aureus* (EMRSA-15) variants detected in healthy and diseased individuals in India. J Med Microbiol 2010;59(7):815–21.
16. Chandel DS, Johnson JA, Chaudhry R, et al. Extended-spectrum beta-lactamase-producing Gram-negative bacteria causing neonatal sepsis in India in rural and urban settings. J Med Microbiol 2011;60(Pt 4):500—507.
17. Jain S, Chowdhury R, Datta M, Chowdhury G, Mukhopadhyay AK. Characterization of the clonal profile of methicillin resistant *Staphylococcus aureus* isolated from patients with early post-operative orthopedic implant based infections. Ann Clin Microbiol Antimicrob 2019;18:8.
18. Struelens MJ, Deplano A, Godard C, Maes N, Serruys E. Epidemiologic typing and delineation of genetic relatedness of methicillin-resistant *Staphylococcus aureus* by macrorestriction analysis of genomic DNA by using pulsed-field gel electrophoresis. J Clin Microbiol 1992;30:2599–605.
19. Cowan ST, Barrow GI, Steel KJ, Feltham RKA. Characters of Gram-positive bacteria. In: Barrow GI, Feltham RKA, editors. Cowan and Steel's Manual for the Identification of Medical Bacteria. Third ed. Cambridge: Cambridge University Press; 2004. p. 52–9.

20. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing CLSI Supplement M100. 29th ed. Wayne, PA; 2019.
21. Centres for Disease Control and Prevention PulseNet. Oxacillin-resistant *Staphylococcus aureus* on PulseNet (OPN): Laboratory Protocol for Molecular Typing of *aureus* by Pulsed-field Gel Electrophoresis (PFGE). Atlanta, GA: CDC; p. 1–24.
22. Murchan S, Kaufmann ME, Deplano A, et al. Harmonization of pulsed-field gel electrophoresis protocols for epidemiological typing of strains of methicillin-resistant *Staphylococcus aureus*: a single approach developed by consensus in 10 European laboratories and its application for tracing the spread. J Clin Microbiol 2003 Apr;41:1574–85.
23. McDougal LK, Steward CD, Killgore GE, Chaitram JM, McAllister SK, Tenover FC. Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national database. J Clin Microbiol 2003;41:5113–20.
24. New England BioLabs Inc. Restriction Enzyme Troubleshooting Guide [Internet]. [cited 2019 Dec 11]. Available from: https://www.neb.com/tools-and-resources/troubleshooting-guides/restriction-enzyme-troubleshooting-guide
25. Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler VG. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev. 2015;28:603–61.
26. Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group I. Methicillin resistant *Staphylococcus aureus* (MRSA) in India: prevalence & susceptibility pattern. Indian J Med Res 2013;137:363–9.
27. Vincent J-L, Rello J, Marshall J, et al. International study of the prevalence and outcomes of infection in intensive care units. JAMA 2009;302:2323–9.
28. Dar JA, Thoker MA, Khan JA, et al. Molecular epidemiology of clinical and carrier strains of methicillin resistant *Staphylococcus aureus* (MRSA) in the hospital settings of north India. Ann Clin Microbiol Antimicrob 2006;5:22
29. Fomda B, Thokar M, Khan A, et al. Nasal carriage of methicillin-resistant *Staphylococcus aureus* among healthy population of Kashmir, India. Indian J Med Microbiol2014;32:39–43.
30. Krishnan PU, Mahalakshmi P, Shetty N. Strain relatedness of endemic MRSA isolates in a burns unit in South India - a five year study. J Hosp Infect 2002;52:181–4.
31. Brown AF, Leech JM, Rogers TR, McLoughlin RM. *Staphylococcus aureus* colonization: modulation of host immune response and impact on human vaccine design. Front Immunol [Internet]. 2014;4:507. Available from: https://www.ncbi.nlm.nih.gov/pubmed/24409186.
32. Vonberg R-P, Stamm-Balderjahn S, Hansen S, et al. How often do asymptomatic healthcare workers cause methicillin-resistant *Staphylococcus aureus* outbreaks? A systematic evaluation. Infect Control Hosp Epidemiol 2006;27:1123–7.
33. Elston JWT, Barlow GD. Community-associated MRSA in the United Kingdom. J Infect 2009;59:149–55.

34. Wilcox MH. Screening for MRSA. BMJ 2008;336(7650):899–900.
35. Chaberny IF, Schwab F, Ziesing S, Suerbaum S, Gastmeier P. Impact of routine surgical ward and intensive care unit admission surveillance cultures on hospital-wide nosocomial methicillin-resistant *Staphylococcus aureus* infections in a university hospital: an interrupted time-series analysis. J Antimicrob Chemother 2008;62:1422–9.
36. World Health Organisation. Global guidelines for the prevention of surgical site infection. Second. Geneva; 2018.
37. Bens CCPM, Voss A, Klaassen CHW. Presence of a novel DNA methylation enzyme in methicillin-resistant *Staphylococcus aureus* isolates associated with pig farming leads to uninterpretable results in standard pulsed-field gel electrophoresis analysis. J Clin Microbiol 2006;44:1875–6.

Tables

Table 1 Criteria for interpreting PFGE patterns [8].

Category	Number of Fragment Differences	Similarity Index
Indistinguishable	0	100%
Closely related	2-3	80%
Possibly related	4-6	50% - 80%
Different	≥ 7	< 50%

Table 2 Antibiotypes of the study isolates.

Antibiotype	Susceptibility	No. of Isolates (*%*)
Ab1	GM, NET, TMP-SMX, TE, E, CD, LZ, VA, TEI	12 *(12%)*
Ab1a	GM, NET, TMP-SMX, TE, E, CD_i, LZ, VA, TEI	2 *(2%)*
Ab2	GM, NET, TMP-SMX, TE, CD, LZ, VA, TEI	8 *(8%)*
Ab2a	GM, NET, TMP-SMX, TE, CD_i, LZ, VA, TEI	40 *(40%)*
Ab2b	GM, NET, TMP-SMX, TE, LZ, VA, TEI	5 *(5%)*
Ab3	GM, NET, TE, E, CD, LZ, VA, TEI	1 *(1%)*
Ab4	NET, TE, E, CD, LZ, VA, TEI	2 *(2%)*
Ab4a[#]	NET, TE, E, CD_i, LZ, TEI	1 *(1%)*
Ab5	NET, LZ, VA, TEI	1 *(1%)*
Ab6	NET, TE, CD, LZ, VA, TEI	2 *(2%)*
Ab6a	NET, TE, CD_i, LZ, VA, TEI	1 *(1%)*
Ab7	NET, CD_i, LZ, VA, TEI	2 *(2%)*
Ab8	GM, NET, TE, CD, LZ, VA, TEI	5 *(5%)*
Ab8a	GM, NET, TE, CD_i, LZ, VA, TEI	3 *(3%)*
Ab8b*	GM, NET, TE, LZ, VA, TEI	1 *(1%)*
Ab9	NET, TMP-SMX, TE, CD, LZ, VA, TEI	2 *(2%)*
Ab9a*	NET, TMP-SMX, TE, CD, LZ, VA, TEI	1 *(1%)*
Ab10	NET, TMP-SMX, TE, E, CD, LZ, VA, TEI	1 *(1%)*
Ab11	GM, NET, CD, LZ, VA, TEI	1 *(1%)*
Ab11a	GM, NET, CD_i, LZ, VA, TEI	2 *(2%)*
Ab12	NET, TMP-SMX, CD, LZ, VA, TEI	1 *(1%)*
Ab12a	NET, TMP-SMX, CD_i, LZ, VA, TEI	1 *(1%)*
Ab13	NET, E, CD, LZ, VA, TEI	1 *(1%)*
Ab14	GM, NET, TMP-SMX, E, CD, LZ, VA, TEI	1 *(1%)*
Ab15	CIP, GM, NET, TMP-SMX, E, CD, LZ, VA, TEI	1 *(1%)*
Ab16	CIP, GM, NET, TMP-SMX, TE, E, CD, LZ, VA, TEI	1 *(1%)*
Ab17	CIP, NET, TE, CD, LZ, TEI, VA	1 *(1%)*

CIP - ciprofloxacin, GM - gentamicin, NET - netilmicin, TMP-SMX - trimethoprim-sulfamethoxazole, TE - tetracycline, E - erythromycin, CD - clindamycin, CD_i – inducible clindamycin resistance, LZ - linezolid, VA – vancomycin, TEI – teicoplanin.

*Intermediate susceptibility to erythromycin. #Intermediate susceptibility to vancomycin.

Figures

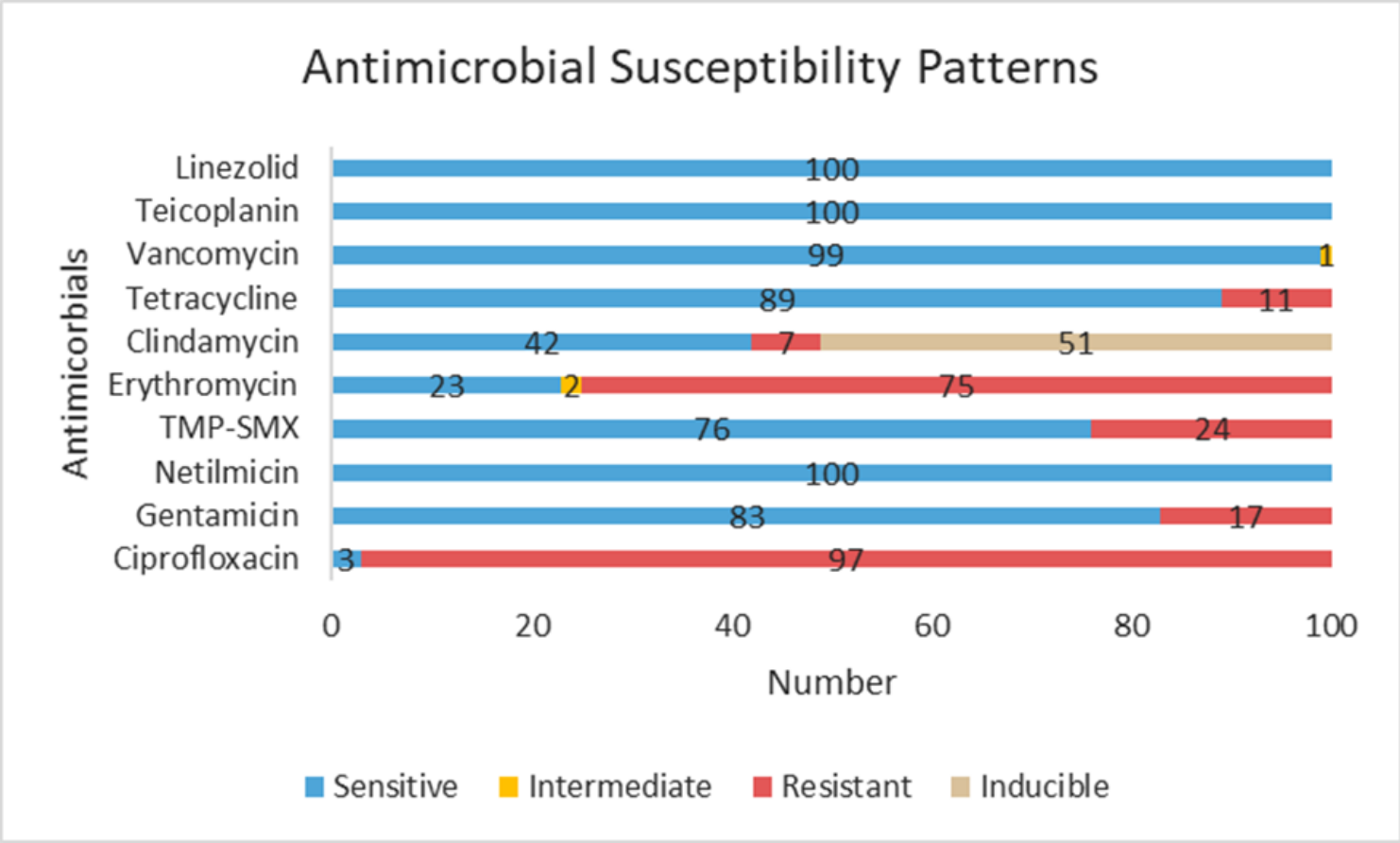

Figure 1

Antimicrobial susceptibility patterns of MRSA isolates.

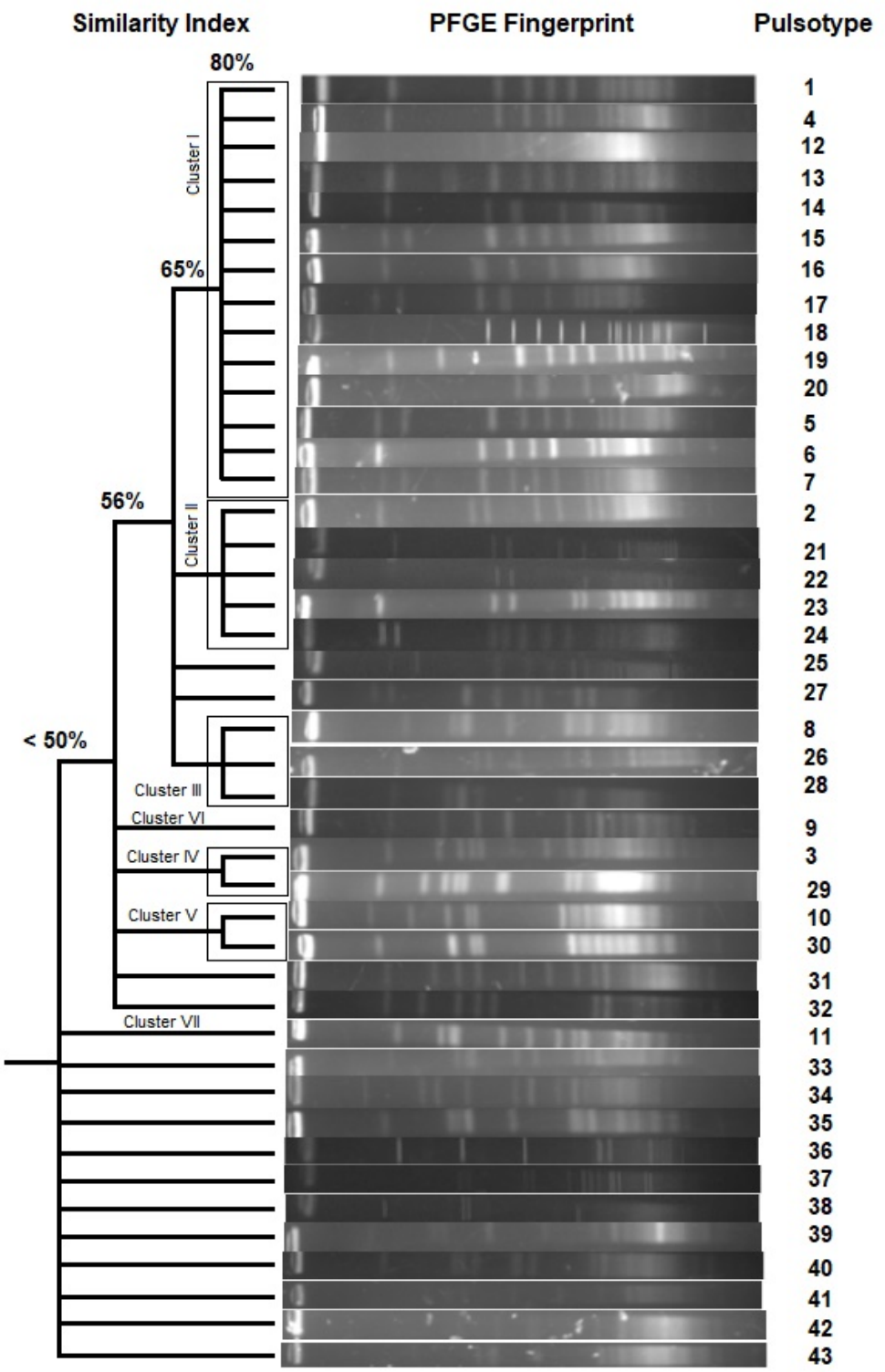

Figure 2

Antimicrobial susceptibility patterns of MRSA isolates.

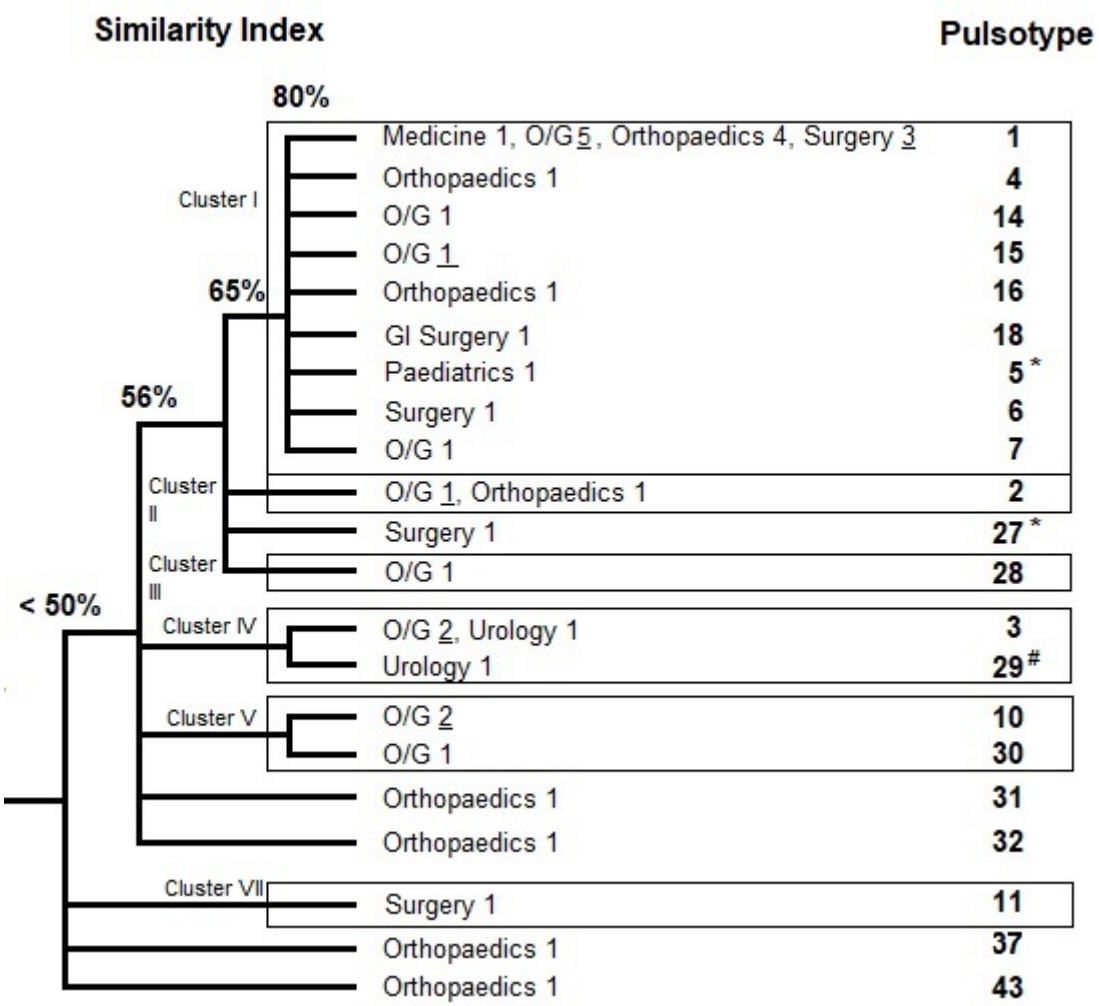

Figure 4

Relationship between isolates obtained from hospital-acquired infections and screening from various disciplines. The underlined figures include isolates obtained from screening specimens. O/G: Obstetrics and Gynaecology. '*' indicates isolate from CLABSI '#" indicates isolate from CAUTI The remaining isolates were all from SSIs.