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WHOLE GENOME SEQUENCING: A TOOL TO MONITOR METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS MECHANISM PREVALENT IN HOSPITAL AND PUBLIC PLACES OF INDIA

Rohit D. Chandekar[1] and Prafulla S. Katkar*[2]
[1]Department of Microbiology, Gramgeeta Mahavidyalaya, Chimur, Chandrapur, MS. India.
[2]Department of Microbiology, Guru Nanak College of Science, Ballarpur, MS. India.
Email: psk.zeal@gmail.com.

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ABSTRACT: Present health care system and public places are experiencing immense pressure of pathogenic attack. Many strains prevalent in these premises are found to be multidrug resistant and reported to be prominent with coagulase negative staphylococci and methicillin resistant Staphylococcus aureus (MRSA). The transmission of these MRSA easily brought about by skin to skin or by aerosol and hence can easily spread via frequently touched public places. India being a highly populated country where its major share of population utilises public transports or public places and government hospitals in general. The chances of easy transmission via these routes are found to be very common and hence surveillance of public and hospital environment is demanded with local authorities so that possible spread could be controlled. To make the study more comprehensive use of whole genome sequencing is recommended to scan the present status of antimicrobial resistance and transmission mechanism at molecular level. We proposed to use whole genome sequencing as a tool to monitor MRSA pathogenicity at community level for better control action in coming time once we check the present drug resistance pattern prevent in strains of MRSA.

Key words: - MRSA, Multidrug resistant, whole genome sequencing.

INTRODUCTION :

The evolution of medical science has done immeasurable good for humanity, but it has also led to some adverse effects one of which is growing into a major, global threat – Antimicrobial resistance (AMR). Over use or rather, abuse of antibiotics over the years has led to this situation where the number of resistant pathogens is increasing exponentially while there is a major void in antibiotic discovery (Speck 2013; Ventola 2015; Zaman et al. 2017). The World Health Organization has predicted nearly 10 million deaths worldwide due to resistant infections by 2050 (WHO 2014).

AMR is causing serious threats to public health globally. AMR is exhibited through several mechanisms that include multidrug efflux systems, antibiotic degrading/blocking enzyme production, outer membrane porins and target mutations. Rampant use of antibiotics has caused a widespread problem of development of antibiotic resistance in bacteria due to horizontal gene transfer as well as de novo mutations (Depardieu et al. 2007; Händel et al. 2014; Martínez and Rojo 2011).

Antibiotics form a major part of modern medicine. Since close to a century, they have helped enable demanding procedures like surgeries, organ transplants, management of immune compromised and cancer patients with decreased mortality rate (M. Hutchings, Truman, and Wilkinson 2019; M. I. Hutchings, Truman, and Wilkinson 2019). Since the 1990s, no new classes of antibiotics have been discovered and theoretically, it also is impossible to develop an antibiotic to which bacteria cannot evolve resistance (Livermore 2004). The World Health Organization (WHO) has predicted that by 2050,

roughly 700,000 deaths will be caused around the world by drug-resistant infections which will also drastically affect the economy (Dadgostar 2019).

Antimicrobial resistance in human pathogens

Growing resistance in ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) pathogens is a looming threat to humankind. The ESKAPE pathogens are a group of six highly virulent, nosocomial pathogens with highest risk of mortality (Mulani et al. 2019; De Oliveira et al. 2020; Rice 2008; Sood and Perl 2016). They display multidrug resistance by commonly three major mechanisms – antibiotic cleavage by enzyme production, antibiotic target site modification, reduced accumulation in the cell by increased efflux or reduced permeability (Munita and Arias 2016). Most of these organisms also biofilms which are extremely resistant structures formed by bacteria to protect themselves from antibiotics (Characklis 1990; Donlan 2002). Antibiotic resistance in addition to biofilm formation capacity has made these ESKAPE pathogens highly resilient. The colonization of biofilm on medical devices, catheters, ventilators and other medical instruments is key to causing the spread of many deadly infections in hospitals (Zheng et al. 2018). Keeping this grave situation in mind, the World Health Organization in 2017 had released a list of priority pathogens for which novel antibacterials needed to be developed (Tacconelli et al. 2018; Tillotson 2018).

One of the leading priority pathogens causing high mortality globally is the methicillin-resistant *Staphylococcus aureus* (MRSA).

Antimicrobial resistance in Staphylococcus aureus

Staphylococcus aureus is a Gram positive, facultative anaerobe commonly causing several skin infections, urinary tract infections, food poisoning amongst other infections (Foster and Foster 1963; Moroni et al. 1996). High level of resistance has been acquired by *S. aureus* over the course of evolution. MRSA strains were broadly differentiated into two groups – hospital-associated MRSA (HA-MRSA) strains and community-associated MRSA (CA-MRSA) strains. Genotypically, the HA-MRSA are characterized by presence of SCC*mec* types I, II and III while CA-MRSA are characterized by presence of SCC*mec* types IV and V. Both these strains have been responsible for high mortality in infected patients, more so in the case of CA-MRSA.

MRSA prevalence in India

There is an increasing prevalence of community acquired MRSA in India. It is now endemic in India and strains have been isolated from all different parts of the country. The prevalence is seen to be ~13-47% in India (Joshi et al. 2013; Patil et al. 2021). Most of the strains possess SCC*mec* IV and SCC*mec* V genes that are typical of CA-MRSA. The emergence of MRSA strains is associated with high morbidity and mortality, prolonged hospital stay and high treatment cost. Poor maintenance of hygiene in hospitals in resource-poor settings, especially crowded ICUs poses a bigger threat of spread of MRSA in developing countries like India.

Typing methods in MRSA identification

The characteristics, transmission routes and host specificity keep evolving and there is a paramount need of constant vigilance and monitoring of the MRSA strains. Understanding the molecular changes in the MRSA is critical for planning appropriate precautionary measures, diagnostics and appropriate prophylaxis. To understand the molecular basis of MRSA, it is important that the major methicillin resistance genes – namely Staphylococcal Cassette Chromosome *mec* (SCC*mec*) genes, its types and

methicillin-resistant gene (*mec*A) and its homologues are studied and properly typed.

Currently, advanced methods including whole-genome approaches are developed for MRSA typing (Figure 1). The typing technique is key in ensuring precise phylogenetic analysis and MRSA lineage. The typing technique should possess the characteristics of high performance / efficacy, convenience, discriminatory power, reproducibility and comparability by global standards (J. Price et al. 2013; J. R. Price et al. 2013).

Some of the popular molecular typing methods are as follows:

1. Multilocus enzyme electrophoresis (MLEE)
2. Multilocus sequence typing (MLST)
3. PCR-based typing – includes amplified fragment length polymorphism (AFLP), random amplification of polymorphic DNA (RAPD) and arbitrarily / repetitive element based PCRS, namely, AP-PCR and Rep-PCR..
4. Pulse-Field Gel Electrophoresis – this technique involves restriction digestion of the bacterial DNA and separation of the fragments by periodically pulsing with electrical field in alternate orientations. This allows for a sharper resolution of the bands that help distinguish between the strains. Of all the different restriction endonucleases, SmaI-based typing is considered to be the gold standard for MRSA typing.
5. Microarrays – microarray analysis is a high throughput method involving the probing of bacterial DNA with a collection of DNA probes that are complementary to particular species/ strain.

Whole genome Sequencing (WGS) of MRSA

WGS is an extremely powerful tool providing the best identification of the genetic diversity in MRSA (J. Price et al. 2013). WGS is the method of analysing genomes based on sequencing them in depth repeatedly over a million times using fast, high-throughput and automated technology (Kuroda et al. 2001; McAdam et al. 2012; Miró et al. 2013; Peleg et al. 2012). Although they are useful in a number of applications, they are routinely used to analyze genetic variations and mutations. The interesting thing about WGS data is that even a single dataset provides a plethora of information apart from just the genotyping and therefore a number of correlations can be drawn and further enriched data can be generated.

Since 2001, whole genomes of a number of MRSA strains have been analyzed in detail and a lot of important information surrounding their resistance has been obtained. The whole genome data has revealed that the MRSA strains are significantly similar to their sensitive counterparts except for the SCC*mec* region. It has also been observed that the resistant strains are highly variable genetically importantly in their regulatory genes and surface receptors (Dendani Chadi et al. 2022). Also, the occurrence of mobile genetic elements (MGEs) show substantial variations even between the isolates of the same lineage. The minor variations revealed within the strains are also observed mostly only in their single nucleotide polymorphisms (SNPs) or MGEs (Lakhundi and Zhang 2018).

WGS in prediction of antibiotic resistance

WGS is useful in understanding the mechanisms of antibiotic resistance as well as predict novel pathways. It also helps in understanding the source of the genes and their evolution and acquisition in *S. aureus*. The WGS confirmed that methicillin resistance in *S. aureus* is also acquired through interspecies transfer of the *mec*A gene from *Staphylococcus epidermidis* (Bloemendaal, Brouwer, and Fluit 2010). It has also been observed that accumulation of point mutations in *S. aureus* over time has reduced its susceptibility to glycopeptides. Vancomycin resistance was acquired from horizontal transfer from

Enterococci (Kos et al. 2012; Levine 2006). Repeated exposure to daptomycin in the clinical setup led to evolution of the phospholipid biosynthesis pathways leading to reduced susceptibility to the antibiotic (Peleg et al. 2012). A study displayed the successful use of WGS method in rapid identification of antibiotic susceptibility in MRSA strains during an outbreak in a neonatal ward. Another study used (Köser et al. 2012) the method effectively in studying the resistance / sensitivity profiles of a panel of 18 genes with antistaphylococcal agents (Nonhoff, Rottiers, and Struelens 2005). The comparison of WGS with common phenotypic methods like Vitek yielded a higher specificity and sensitivity (>95%) with WGS further promoting its application as frontline screening method.

Robust bioinformatic tools help with instant identification of resistance genes in sequenced isolates. An entire database of the 'resistome' can be created using WGS for such isolates and evaluated.

Virulence prediction using WGS data

WGS data has been crucial in revealing varying degrees of virulence in MRSA. Several genetic determinants of virulence have been analyzed using genome-wide association studies. About 70 novel candidate genes determining virulence factors have been discovered earlier in *S. aureus* genomes (Kuroda et al. 2001). Additionally another 19 virulence factors were identified and persistently are still being discovered (Baba et al. 2002). In addition to just determination of the virulence gene, WGS data helps in understanding the type of the virulence factor i.e. whether it is a toxin gene or antibiotic resistance gene and in how many copies they are present in the genome (Köser et al. 2012; McAdam et al. 2012).

Surveillance and MRSA population studies using WGS

With WGS now made extremely efficient, handy and quick with even benchtop or palm-sized devices available, they offer a great opportunity for use in surveillance of MRSA locally as well as globally. WGS can give great accuracy in providing early warning for emerging pathogenic strains of MRSA (Harris et al. 2010; McAdam et al. 2012).

Importance of WGS in infection control in hospitals

Routine monitoring of various hospital samples including patients, healthcare workers, hospital environment, medical devices and staff by sequencing can give invaluable information regarding transmission of MRSA in hospitals.

CONCLUSION :

WGS is an excellent tool in all respects for MRSA isotyping, surveillance and infection control. A single sequencing data can give an immeasurable amount of information about the various MRSA strains from their origin or source to predicting their future evolution. However, there still are a number of obstacles to overcome before WGS can become mainstream in MRSA typing. The biggest hurdle is requirement of significant bioinformatic expertise for analysis of WGS data.. With further development of softwares supporting WGS, the method will soon see easier acceptability and wider applications. Progressive characterization of the genetic diversity over time will yield more reference genomes leading to better success with the WGS data for MRSA typing.

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Figure 1: Different methods used in MRSA isotyping.