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MITIGATION STRATEGIES TO REDUCE BURDEN OF ANTIBIOTIC RESISTANCE: CASE STUDY OF *STAPHYLOCOCCUS AUREUS*

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AUTHOR'S CONTRIBUTIONS

The work is a product of the intellectual environment of the whole team; and all the authors have contributed in various degrees towards the conceptualization and designing of the manuscript. RC, AC, RN and PT have made substantial contributions to conception and design, acquisition of data, analysis and interpretation of data; AN, RG and NS have helped in drafting the article and reviewing it ; revising it critically for important intellectual content; RA and RKS have reviewed the article and have given final approval of the version to be published.

ABSTRACT

Staphylococcus aureus infections remain a leading cause of morbidity and mortality in health care settings and community despite a long history of association. It is generally held responsible for causing considerable economic losses, estimated to be around 2-4 billion dollars per annum, owing to longer hospital stays, expensive 2nd/ 3rd line antibiotic treatment etc. Although stringent hospital hygiene regime has checked the spread of Hospital associated Methicillin Resistant *Staphylococcus aureus* (HA-MRSA) by 37%, Community-associated MRSA (CA-MRSA)

strains spread remains unhindered. The lack of specific risk factor for CA-MRSA and gradually increasing infection rate of 17% has alarmed the scientific community. The genetic flexibility, owing to a large resistome at hand and adaptability of this pathogen has favoured the emergence of multi drug resistant strains, limiting therapeutic options and delaying vaccine development. Thus, an alternative method is imperative to halt its destructive progression. The present study focuses on conceptualizing an alternative herbal mitigation strategy utilizing mechanistic approach to nip this menace in the bud. These alternatives, due to associated holistic approach, provide an edge over other existing candidates by simultaneous action on multiple non-essential factors, thereby hindering the pathogens disease establishment capabilities, without enhancing selection pressure on microbial populations.

Keywords: Multi-Drug Resistant *Staphylococcus aureus,* Herbals, Antibiotic resistance, Superbugs, Ethnopharmacology, Nosocomial infections

INTRODUCTION

The rampant usage of antibiotics as prophylactics, general non compliance to antibiotic treatment regimes and discordant antibiotic therapy, has led to the selective emergence of human pathogens with drug resistance capabilities. Other contributing factors include misuse of antibiotics as growth supplements for livestock, extensive usage in agriculture and aquaculture and commercialization of antiseptic household and sanitation products **[1-3]**. This has accelerated the emergence of multi drug resistant (MDR) organisms to epic proportions, increasing the attrition rates of available antibiotics, even threatening the life expectancy of novel antimicrobial entries to the market by generating a constant selective pressure promoting MDR organism's deliberate evolution **[4]**.

The situation is best exemplified by the emergence of MDR infectious diseases with the highest annual death toll like acute respiratory infections, gastrointestinal infections/diarrheal diseases, malaria, AIDS, measles, tuberculosis, and other infectious diseases commonly referred to as nosocomial infections. These diseases together account for about 90-95% of mortality from infections reported worldwide, the second leading cause of death despite the availability of antibiotics or other treatment regimes **[5]**. Often the treatment cost of MDR infections is increased by 30-100%, considering the cost of second line antibiotics, longer hospital stays and subsequent recovery periods, etc. This makes treatment itself an option available for a

privileged few **[6, 7]**. Hospitals have now become a niche for MDR organisms providing a perpetual selection pressure targeting immuno- compromised/suppressed patients, majority of whom have recently undergone invasive procedures. The health sector has been most affected by this growing menace and as per an estimate incurring losses to the tune of 2-7 billion dollars per annum in US and UK **[8]**. Its impact is limitless in developing countries which exhibit higher mortality rates due to unavailability/unaffordability of treatment regimes **[9, 10].** Increased incidences of nosocomial MDR infections such as bloodstream infections, surgical wound infections, urinary tract infections, respiratory infections and gasteroentiritis, primarily caused by Gram positive (coagulase-negative *Staphylococci*, *Staphylococcus aureus*, *Enterococci spp.*) and Gram negative bacteria (*Acinetobacter baumannii*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter species*, *Serratia marcescens*) have been reported **[11, 12]**.

In addition, the biothreat potential of MDR organisms should not be undermined. The ease of acquiring these agents and utilization of dual use technologies, readily available at ubiquitous private/public research institutes as well as in diagnostic laboratories, for its genetic alteration, possibly targeting microbial virulence, drug resistance and dispersal methods makes it a viable option for non-state agents for malicious intent. Since the regulation of dual use technologies still remains in the grey area of public debate other alternative preparative methods must be undertaken. However, the presence of a large arsenal of developed antibiotics and herbal agents greatly diminishes the lucrativity of such an act. Continued efforts targeting holistic mitigation of MDR infections are in order.

In a time where on one side, an increasing number of drug resistant strains are emerging hitherto and on the other, a rapid decline in the introduction of novel drugs targeting molecular mechanism poses an urgent need for judicious use of existing antibiotics and bioprospection for new alternative mitigation strategies from natural flora. This review presents a study of the eras of microbial evolution towards multi drug resistance with *Staphylococcus aureus* as a specific case study. The historical evolution complementary to human advancements with existing dynamically evolving strategies employed have been discussed. The alternative mitigation strategies being explored focusing on compounds of herbal

origin, with their benefits and limitations have also been included.

Eras of Microbial Evolution Towards Drug Resistance (Figure 1)

Pre-Antibiotic Era [till 1908]

Before the discovery and worldwide application of antibiotics to treat infectious diseases folk remedies was the mainstay in societies. Many such remedies were based on consumption/application of concoctions made of various native herbs/ plants, crushed minerals/soils and animal products **[13]**. For example, in 17th century, the empirical treatment of bubonic plague in Britain included the use of Theriac (multi component herbal medicine popular for treatment and prevention of all diseases.), crushed grains of bezoar (mineral stone) and powder of a unicorn's horn albeit little relief **[14]**. Some effective treatments included cinchona bark extract for the treatment of malaria, extensively used in Europe in 17th century, ipecacuanha root for the treatment of dysentery in South America and the elaborate traditional herbal practices of India (Ayurveda) and China (Traditional Chinese Medicine), some of which, having withstood the test of time, are still in popular demand. Other methods employed were spiritual in nature and being of little use were dangerous at times (common historic practice of bloodletting to treat diseases). Therefore the severity of infectious diseases was at an all time high, the poor standard of living also contributed to the high transmission of infectious diseases **[13]**.

Various eras of microbial evolution towards drug resistance is depicted in **Figure 1**.

Penicillin Era [1908-1945]

The first successful application of a drug as an antimicrobial was made possible by Paul Ehrlich's team with the synthesis of Salvarsan in 1908 for the treatment of Syphilis **[15]**. This discovery lead to the beginning to the Penicillin Era, fuelling research in the discovery of novel antimicrobial compounds (sulfonamides, penicillin and streptomycin). An important hallmark of this era was Second World War which resulted in mass production and extravagant usage of antibiotics to aid in warfare casualty treatment. Thus a sharp fall in severity of infectious diseases was witnessed. It could be primarily attributed towards availability of the most important and effective antimicrobial drugs i.e., penicillin in 1941 and streptomycin in 1944 **[16]**. However, the extensive use of penicillin lead to the emergence and spread of Penicillin Resistant *Staphylococcus aureus* (PRSA) in mid 1940's **[17, 18]**. The wartime logistic activities further aggravated the spread of PRSA across international borders.

Recovery Era [1945-1950]

Following the World War, the world exhibited a sign of relieve. There was an increase in the number of health care facilities and standard of living was also improved, thereby reducing the severity and incidence of infectious diseases. The discovery of novel classes of antimicrobial agents had delivered two important broad action spectrum agents, Chloramphenicol and Tetracycline in 1948 which are used till date despite associated adverse reactions **[19, 20]**.

Post Second World War Era [1950-1975]

Finally in post world war era, with antimicrobial discovery and research at its hilt, equipping the health care sector with potent medicines a further dip in severity of infectious disease was witnessed. This era saw the performance of the very first organ transplant directed by Dr. Lawler at Little Company of Mary Hospital. A shift in food habits was also witnessed indicated by commercial use of Food irradiation and chemical for preservation to sustain flourishing food storage and processing industries. The development of commercial aviation was pivotal in the increase of global travelling and hence spread of exotic microbial agents. Another highlighting feature of this era was the Green Revolution from 1940-1970 which triggered extensive use of pesticides, herbicides and chemical fertilizers in agriculture. All this contributed to the development and spread of resistance genes, especially emergence of Zoonosis diseases was witnessed as world level pandemic, indicating a frequent interaction of microflora in various habitats leading to active re-assortment enhancing virulence. Among flu pandemics, the world witnessed the first Spanish Influenza pandemic claiming the lives of millions. Important drug resistant organisms of this era were Methicillin Resistant *Staphylococcus aureus* (MRSA) in 1961, Penicillin Intermediate Streptococcus pneumonia (PISP) in 1967 and Penicillin Resistant Haemophilus influenzae (Penicillinase producing strains) **[21-23]**. It could be visualized as a dawn of antibiotics as the first line of medical emergency management tool since the resistance among pathogens steadily increased.

Modern Era [1975-2000]

Little progress was made in this era in discovery of novel classes of antimicrobial drugs. The growing severity of infectious diseases was then targeted by improving hygiene and sanitation conditions, implying greater usage of antibiotic soaps, chemical cleaning agents, enzymatic detergents etc, however, promoting further emergence and

re-emergence of infectious diseases with varying resistance/associated virulence. A logical explanation of this shift lies in the drastic increase in hospitals and health care facilities. This led to generation of substantial biological waste, which in turn demanded an improvement in the waste management capabilities. This provided a breeding ground for human pathogens providing ample opportunities for horizontal gene transfer and emergence of MDR strains **[24]**. Advances in various fields of sciences also contributed to the said cause by triggering development and application of new technologies like widespread commercial use of UV radiation in water purification systems, chlorination in water treatment/sewage treatment plants, increased usage of chemical food preservatives, food irradiation and refrigeration, use of artificial/chemical ripening of natural food products to feed an ever growing human population as well as, increased usage of antibiotics in animal feeds and agriculture.

In brief, significant increase in the Chemical, Biological, Radiological and Nuclear (CBRN) waste led to environmental pollution and therefore increased mutation rates among fast evolving prokaryotes. Moreover the ever increasing demands of human population had led to mass deforestation, initiating changes in microbial niche with respect to speciation and evolution of mutated species targeting natural hosts itself. This led to increased incidences of zoonosis and reverse zoonotic diseases **[25]**. Thereby deliberately selecting for the most virulent and resistant strains to survive and flourish. Some examples of MDR strains emerged during this time were Penicillin resistant Streptococcus pneumonia (PRSP) in 1977, β-lactamase Negative Ampicillin Resistant (BLNAR) Haemophilus influenzae in 1980, Extended Spectrum β-lactamases (ESBL) producing gram neagative bacilli in 1983, Vancomycin Resistant Enterococci (VRE) in 1986, extensively virulent Community associated Methicillin Resistant *Staphylococcus aureus* (CA-MRSA) in 1990 and Drug resistant Neisseria gonorrhoeae in 1992 **[22, 23, 26]**. This era exhibited a drastic increase in the incidences assocoated with infections involving Drug Resistant Microbial strains such as MRSA, PRSP, BLNAR, etc **[27-29]**.

Biotechnology Era [2000-till date]

This era, hall marked by the sequencing of the Human Genome Project, has witnessed substantial technological growth, especially in Life Sciences and Information Technology. This was made possible by the constant endeavor of the Scientific Community by developing novel techniques and

methodologies to manipulate the microbiota for the human benefit. But these tools themselves have contributed to the 'gene pollution' leading to an increase in antibiotic resistance. The culturing and transportation of Highly virulent Biothreat agents in sophisticated BioSafety Laboratories, in particular, and infectious agents, in general for experimentations/vaccine developments and/or diagnosis has in itself created a very fragile environment where the entrance of an inevitable human error could prove to be very fatal. This era has till date delivered some of the most drug resistant strains such as Vancomycin Resistant *Staphylococcus aureus* (VRSA) in 2002 and Multi Drug Resistant Mycobacterium Tuberculosis in 2005 **[30, 31]**. The present scenario exhibited a steady increase in the severity of infectious diseases and a diminishing arsenal of antimicrobial drugs to counter it. Such era might end with new advancement of molecular medicines by 2015.

Molecular Medicine Era

The current progress in the elucidation of various cellular and molecular mechanisms will, in future, surface the path to pharmacogenetic/ pharmacogenomic based therapy eradicating the elusive predicament of adverse drug reactions **[32]**. It would provide individualized treatment of patients, accounting for inter-individual drug response variability, thereby granting enhanced infection mitigation **[33]**. This would also in turn reduce the incidences of emergence of drug resistant microbes, predictably bringing the emergence rate to base level. The role of nano-technology based treatment will be streamlined and pivotal in this era, enhancing bio-availability of active constituents, possibly reviving obsolete/discarded drug candidates and mitigating pathogens with strain specific thermal ablated nanovectors **[34]**.

The myriad of information gathered through the previous eras will coalesce together harnessing new technologies, promoting human health without disrupting the delicate balance of nature. Here our enhanced understanding of host-pathogen interactions previously gained, giving us an insight to diverse signaling pathways and physiological targets, paves a way for the development of novel antibiotic classes. This approach is in contrast with its predecessors which primarily targeted essential pathways/enzymes conserved with the bacteria for growth such as cell wall synthesis inhibitors (β-lactam antibiotics), protein synthesis inhibitors (macrolides, tetracyclines, aminoglycosides antibiotics) or interfered with cells other constitutive functions/ antimetabolites

(sulfonamides and quinolones) **[35]**. This created a pressure for the selection of strains which resisted the action of these drugs either by producing enzymes which deactivated the drugs or by restricting the entry of the drug molecule inside the cell by over expression of MDR pumps/ cell morphology changes or by changing the drug target itself or by obtaining a compensatory mechanism/activity which nullifies the effect of drug-target interactions **[36]**.

Theoretically by targeting virulence factors the selection pressure is removed from the invading pathogens as their inhibition does not hamper the growth of the microbes but it diminishes its survivability in the hostile host environment, resulting in host immune system mediated clearance of the invading pathogens. This theory is further supported by growing scientific evidence, best exemplified by *Staphylococcus aureus* as a case study discussed in subsequent sections.

***Staphylococcus aureus*: Infectious Journey So Far**

Staphylococcus aureus merits special attention due to its ease of acquisition of resistance against commonly used antimicrobials, referring to lessons learnt from past and evident by the emergence of MRSA in 1961 and Vancomycin Resistant *Staphylococcus aureus* (VRSA) in 2001, eliminating β-lactam antibiotics and vancomycins once prominent role, respectively, in treatment of *Staphylococcus aureus* infections **[37, 38]**. The dwindling number of clinicians armamentarium against this common pathogen is a matter of concern, which is further exaggerated by the attrition rate of available antibiotics and the retardation in commercialisation of novel therapeutic agents **[39]**.

Being a commensal and opportunistic microorganism, it has duly earned the repute of a noteworthy human pathogen. About 20-30% of the human population is permanently colonized with *S.aureus*, with considerable variability primarily due to occupation, geographic, genetic and health/lifestyle factors **[40, 41]**. The major sites of colonization include anterior nares, axilla, perineum, vagina and skin **[42]**. Colonization by *S.aureus* increases the risk of infection thereby rendering certain populations at higher risk **[43]**.

Spread and Severity

Immuno-compromised/suppressed populations are gravely effected by *S.aureus* infections, especially those suffering from diabetes, AIDS and patients having undergone invasive surgical procedures **[44]**. This makes *S.aureus* the most notorious nosocomial pathogen, with MRSA being

responsible for nearly 60 percent of ICU infections **[45]**. This dreadful menace spreading worldwide at a significantly rapid rate is responsible for multiple outbreaks in various levels of health care settings both in developed and developing countries **[46-48]**. Since 1990s, it has even extended its realm to communities having no prior known risks, however symptomology ranges from minor skin/soft tissue infections even to fatal necrotizing infections in severe cases **[49]**.

The previously accepted terminology is location specific being either Hospital Associated MRSA (HA-MRSA) or Community Associated MRSA (CA-MRSA), however the same has been challenged by various confounding reports of CA-MRSA strains in nosocomial outbreaks and vice versa **[50, 51]**.

In the present scenario, this leading killer, posing a momentous health burden even managed to transcend international borders **[52, 53]**. The spread and persistence of pandemic MRSA clones, namely Iberian, Brazilian, Hungarian, New York/Japan and Pediatric MRSA clones bears a testimony to this fact. For example the Iberian Clone spread and persisted in the major part of Europe in the 1990's and subsequently was also detected in USA **[54]**.

This is usually accompanied by the impeding recombination of HA-MRSA strains, harboring resistance to multiple drugs/antibiotics, along with CA-MRSA strains, containing novel virulence with significant health impact could not be ruled out **[55]**. Inspite of such increasing trend the choices of therapeutics is getting limited.

Therapeutic Evolution

The current available treatment options, including antibiotics and surgical interventions have got both cost and risk factors associated with it. Table I enlists some important antibiotics such as Linezolid, Daptomycin, etc; which are currently offering therapeutic regimes against MRSA. However among these 14 antibiotics 8 have been found to be resisted by some strains of MRSA in isolated cases merely after 1 year of their introduction. In addition, it has been observed that these therapeutic regimes are of little use as MRSA continually evolves due to non-judicial use of such regimen.

The scarring associated with surgical interventions and the increased risk of acquiring super infections of hospital origin could not be ruled out.

Based on the analysis of the mode of action of MRSA, the numerous virulence factors, their associated genes and corresponding functions provides an insight into new targets that are

required to be targeted by novel categories of synthetic/herbal therapeutic regimens. MRSA virulence factors may be classified in the categories of Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMM); factors involved in Nutrient Acquisition; Exotoxins; MDR Efflux pumps; Invasins; Immuno-modulatory factors and factors associated in Biofilm formation. Their synchronous synthesis and strict regulation is of paramount importance in establishing MRSA infection.

As depicted in **Figure 2**, Colonization, the determining factor of future MRSA infection, is majorly dependent on the virulence factors for adhesion (EBP, Teichoic acid), competition with prevalent microflora (Bacteriacin) and surviving in the hostile host environment (Lipase, catalase). Tissue invasion is facilitated by adhesion virulence factors and factors which degrade the Extra Cellular Matrix (Hyaluronate, thermonuclease, Staphopain) after an external force/trauma breaches the primary barrier (eg skin, mucosal membrane) granting *S.aureus* access to the host. Following host immune activation, immune modulatory virulence factors (PSM, SE's, TSST, etc) are secreted which impairs host immunity leading to disease establishment giving rise to Staphylococcal Abscess Community (SpA, vWbp, Coa, Isd family) and biofilm formation (Bap, PIA, FnBP, etc). Once the Staphylococcal population reaches its optimal concentration virulence factors promoting dissemination are secreted resulting in multi focal infection/ bacteremia.

Given the redundancy and overlap of function between various virulence factors the following were selected for their therapeutic potency:

(1) Staphyloxanthin; (2) Iron-responsive surface determinant protein B (IsdB); (3) Extracellular adhesion protein (Eap); (4) Extracellular fibrinogen-binding protein (Efb); (5) von Willebrand factor-binding protein (vWbp); (6) Staphylococcal Complement Inhibitor (SCIN); (7) Clumping factor A (ClfA);

The above factors were chosen for their reported activities in mitigating infection either via direct inhibition or by antigen mediated host immune response **[47]**.

This necessitates the presence of an alternative mitigation strategy, which should be safe, effective and at the same time should reduce the probability of pathogens acquiring resistance against it attributed towards its comprehensive mode of action. This strategy would provide us with an appropriate window of opportunity to target the crucial time lag

between vaccine development and spread of a new bio-agent or a novel drug resistant strain utilizing different mode of spread leading to significant socio-economic disruption. For example, if novel H1N1 undergoes recombination with H5N1 the resultant strain will be extremely lethal and virulent, exhibiting high mortality and morbidity.

The spontaneous evolution and emergence of such recombinant pathogens is axiomatic in the present day. Making populations vulnerable to native microflora replete with non native virulence factors. Therefore the delayed response, implying to antibiotic therapy, laboratory diagnosis, vaccine development/administration to infection requires broad spectrum safe bio protective agents.

The virulence factors may prove to be potent targets for novel anti microbial compounds. Recent reports support this and show considerable success in animal models/trials pointing towards the development and subsequent commercialization of antimicrobial compounds targeting virulence factors in near future **[58-61]**.

MRSA with its wide range of virulence factors ranging from Microbial Surface Components recognizing Adhesive Matrix Molecules (MSCRAMMs), to a wide variety of exotoxins and multi drug resistance (MDR) pumps, are primarily responsible for disease outbreaks and severity, and steadily increasing drug resistance spectra. These could be selectively targeted thereby mitigating the threat from this age old adversary utilizing mechanistic approaches. MRSA's disease progression clearly points towards immune evasion and disease establishment stages as the leverage, which could be exploited for changing the course of the infection/disease.

Recent exploration in this domain is evident by the ongoing research in the field of vaccine development. A comparative list of vaccine under trial with their intended targets is provided in Table II. The main factor complicating development of an FDA approved vaccine against *Staphylococcus aureus* is its virulence factor diversity and redundancy, which often presents varied clinical presentations. The challenge of a universal vaccine providing sufficient immuno-protection without compromising safety issues remains an undaunted task ahead.

Future Perspective

Herbal informatics is a novel systematic approach that allows focused herbal drug discovery with an ease at an enhanced pace. MRSA, with a significantly higher virulence, could be selected as a prime target to validate

this approach. The pre-requisite need for the selection of new ligands / herbals against newly evolving pathogens is the development of an e-herbal archive. Presently, herbal research is facing a primary challenge of batch-to-batch variation and non-reference efficacy linked data. Figure 3 depicts a systematic flow to target Multi-Drug Resistant / Biothreat agents and optimally bridging the gap of vaccine development with alternative mitigation strategies. Rapid advances in the information technology can automate the system to design and develop newer formulations exhibiting a broad spectral range against biothreat agents, e.g. Methicillin Resistant *Staphylococcus aureus*.

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All the authors have equally contributed towards the conception and designing of the manuscript. The authors declare no competing financial interests.

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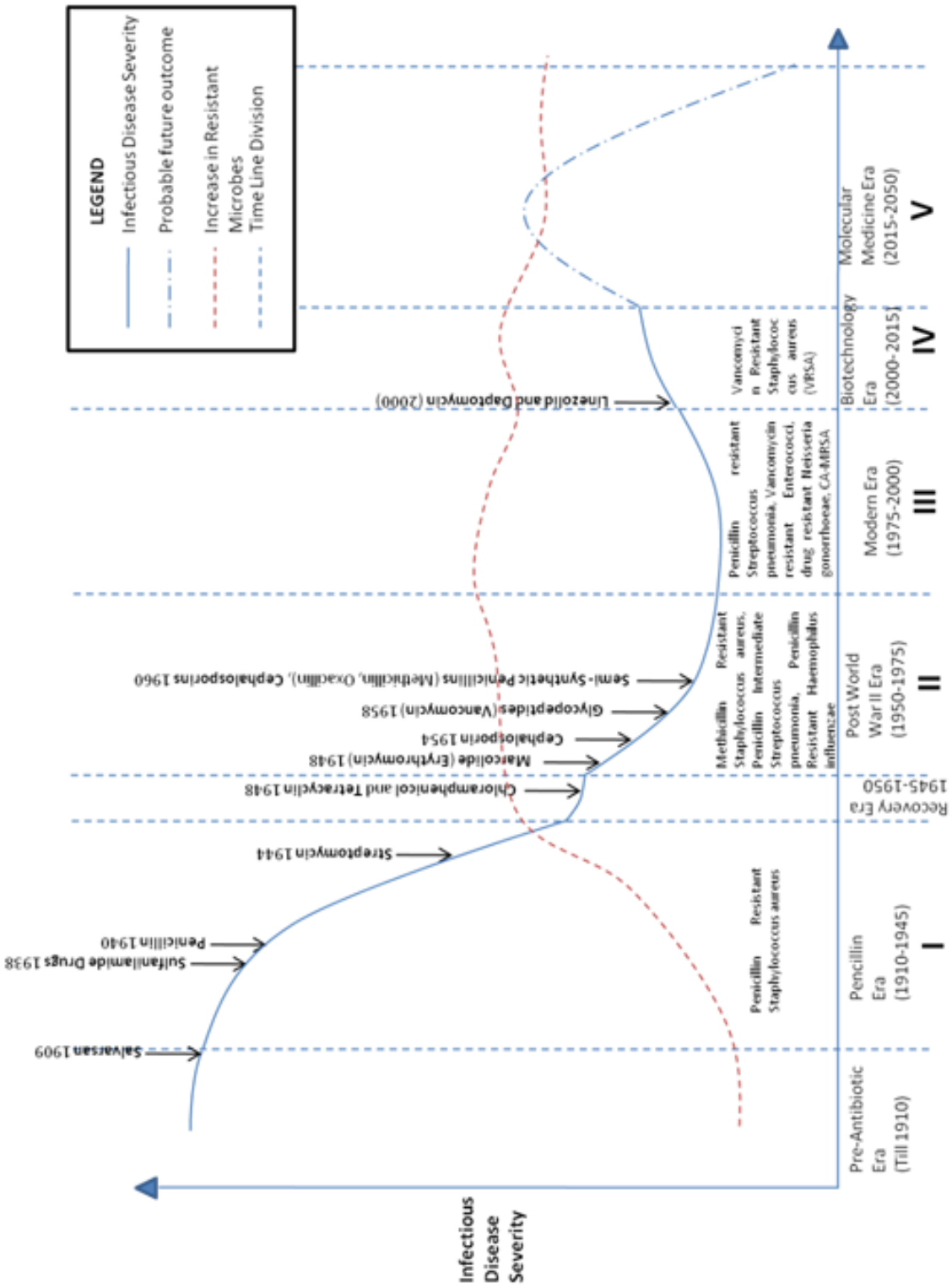

Figure 1: Eras of Microbial Evolution towards Drug Resistance

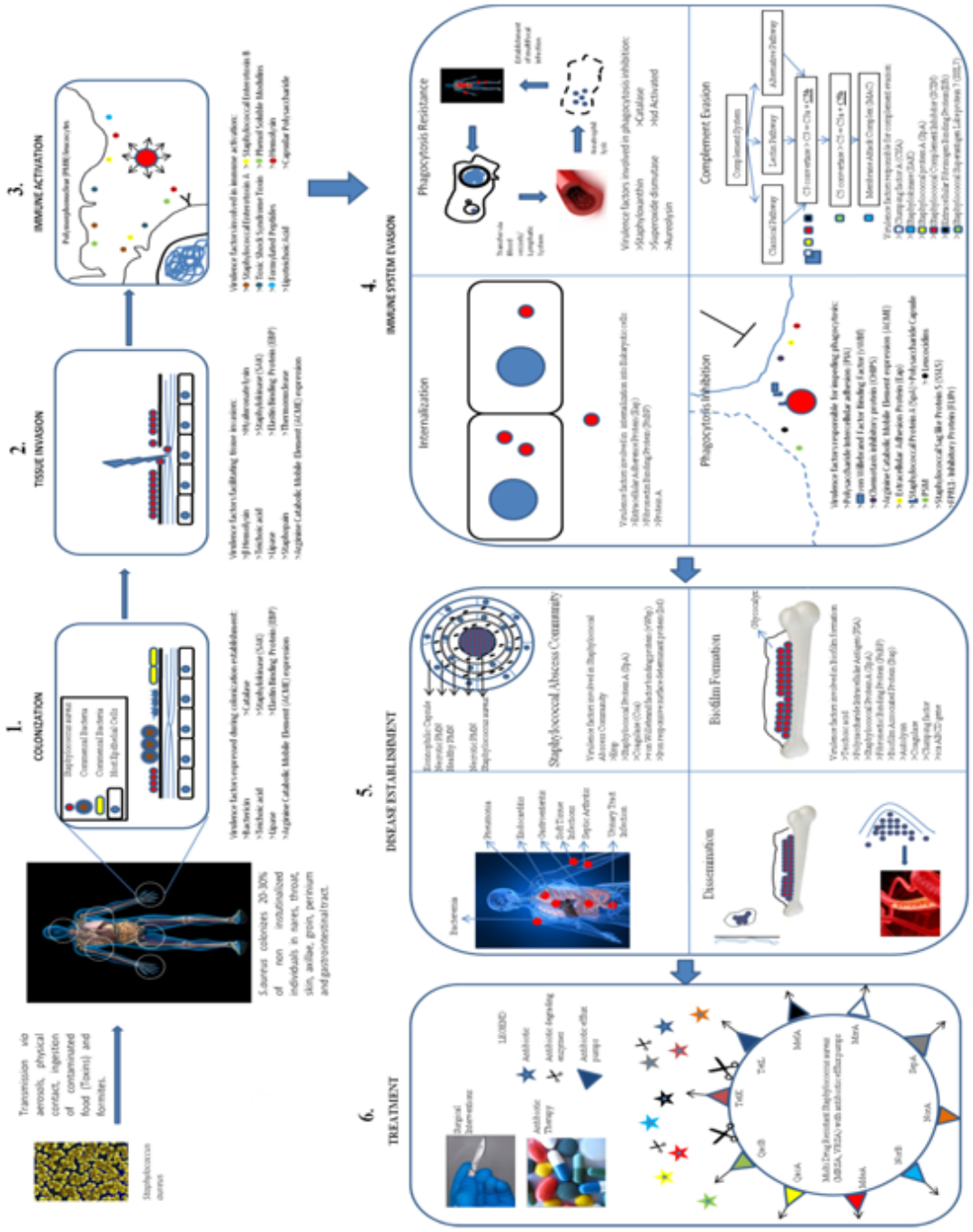

Figure 2: The disease progression of Methicillin Resistant *Staphylococcus aureus* (MRSA) elaborated in following steps: 1.Colonization (Bactericin, Catalase, Teichoic acid, Staphylokinase, Lipase, Elastin Binding Protein, Arginine Catabolic Mobile Element expression); 2. Tissue Invasion (β Hemolysin, Hyaluronate lysin, Teichoic acid, Staphylokinase, Lipase, Elastin Binding Protein, Staphopain, Thermonuclease, ACME expression); 3. Immune activation; (Staphylococcal Enterotoxin A, Staphylococcal Enterotoxin B, Toxic Shock Syndrome Toxin, Phenol Soluble Modulins, Formylated Peptides, Hemolysin, Lipoteichoic Acid, Capsular Polysaccharide) 4. Immune Evasion (Staphyloxanthin, Catalase, Superoxide dismutase, Isd, Aureolysin, Extracellular Adherence Protein, Fibronectin Binding Protein, Staphylococcal Protein A, Clumping factor A, Staphylokinase, Staphylococcal Complement Inhibitor, Extracellular Fibrinogen Binding Protein, Staphylococcal Superantigen Like protein 7, Polysaccharide Intercellular adhesion, von Willebrand Factor Binding Factor, Chemotaxis inhibitory protein, Arginine Catabolic Mobile Element expression, Polysaccharide Capsule, PSM, Leucocidins, Staphylococcal Sag like Protein 5, FPRL1- Inhibitory Protein); 5. Disease Establishment (Emp, Staphylococcal Protein A, Coagulase, von Willebrand factor binding protein, Iron responsive surface determinant protein, Teichoic acid, Polysaccharide Intracellular Antigen, Staphylococcal Protein A, Fibronectic Binding Protein, Biofilm Associated Protein, Autolysin, Coagulase, Clumping factor, ica ABCD gene); 6. Treatment (TetK, TetL, MefA, MsrA, NorA, NorB, MdeA)

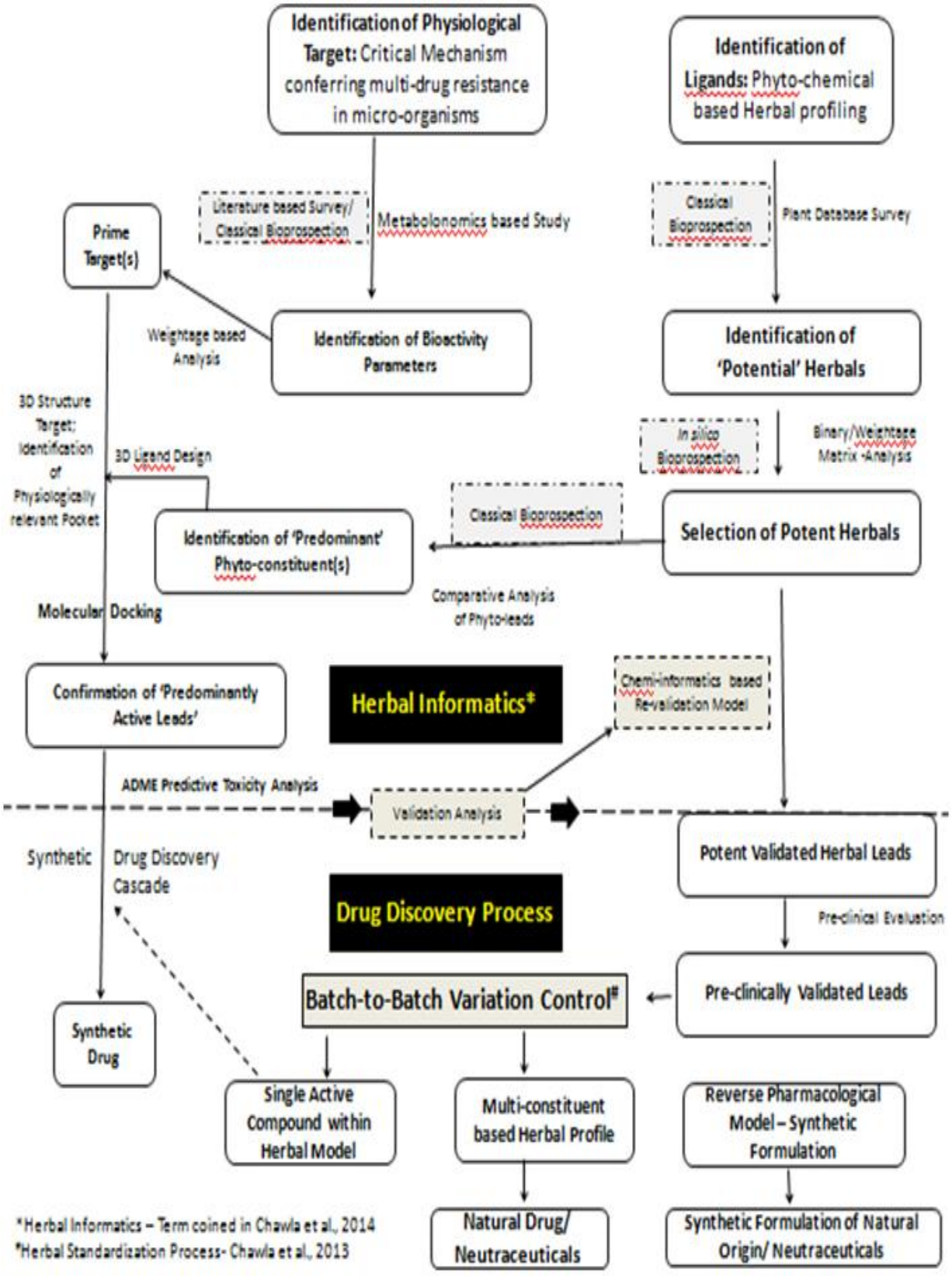

Figure 3: Systematic flow to target Multi-Drug Resistant / Biothreat agents

Table 1: Current antibiotic regimen for treatment of MRSA						
Antibiotics†	**Family**	**Mode of Action**	**Primary Indicators**	**Toxicity**	**MIC**	**Resistance detected in MRSA**
Vancomycin	**Glycopeptide antibiotic**	**Inhibits cell wall synthesis**	**Pneumonia Skin/Soft tissues Bacterimia**	**Nephrotoxicity Thrombocytopenia**	**≤2 µg/ml**	**Yes**
Linezolid	**Oxazolidinones**	**Protein Synthesis Inhibitors**	**Pneumonia Skin/Soft tissues**	**Myelosuppression Lactic acidosis Peripheral and optic neuropathy Serotonin Syndrome**	**≤4 µg/ml**	**Yes**
Tigecycline	**Glycylcycline**	**Protein Synthesis Inhibitors**	**Skin/Soft tissues Intra-abdominal**	**Nausea Vomiting Photosensitivity**	**≤0.5 µg/ml**	**Yes**
Daptomycin	**Lipopeptide antibiotic**	**Causes rapid depolarization of bacterial cell membrane**	**Bacterimia Skin/Soft tissues**	**Muscle toxicity CPK elevation**	**≤1 µg/ml**	**Yes**
Quinupristin/dalfopristin	**Streptogramin**	**Protein Synthesis Inhibitors**	**Skin/Soft tissues**	**Phlebitis Arthralgia and myalgia**	**≤1 µg/ml**	**Yes**
Ceftobiprole	**Fifth generation cephalosporin**	**Inhibits cell wall synthesis**	**Skin/Soft tissues**	**Allergic reactions**	**≤4 µg/ml**	**-**
Ceftaroline	**Fifth generation cephalosporin**	**Inhibits cell wall synthesis**	**Skin/Soft tissues Pneumonia**	**Allergic reactions**	**≤1 µg/ml**	**-**
Dalbavancin	**Second-generation lipoglycopeptide antibiotic**	**Inhibits cell wall synthesis**	**Skin/Soft tissues**	**Nausea Vomiting**	**≤1 µg/ml**	**No**
Oritavancin	**Lipoglycopeptide**	**Inhibits cell wall synthesis and Cell membrane interaction/disruption**	**Skin/Soft tissues**	**Nausea Vomiting**		**-**
Telavancin	**Glycopeptide antibiotic**	**Inhibits cell wall synthesis and Cell membrane depolarization**	**Skin/Soft tissues Pneumonia**	**Renal thrombocytopenia**	**≤1 µg/ml**	
Rifampin		**Inhibition of DNA-dependent RNA polymerase, leading to a suppression of RNA synthesis and cell death.**		**Nausea Vomiting and Unconsciousness Hepatotoxicity and Nephrotoxicity**	**≤2 µg/ml**	**Yes**
Mupirocin	**Monoxycarbolic acid**	**Selective binding to bacterial isoleucyl-tRNA synthetase,**	**Skin**	**NA**	**≤4 µg/ml**	**Yes**
Teicoplanin	**Glycopeptide**	**Teicoplanin inhibits polymerization of cell wall components in susceptible bacteria**	**Bacterimia**	**Ototoxicity**	**≤1.5 µg/ml**	**Yes**
Fosfoxycin	**Phosphonic acid derivative**	**Irreversibly inhibits enolpyruvate transferase (MurA), which prevents the formation of N-acetylmuramic acid**	**Soft tissue infections, Urinary Tract Infections**	**Diarrhea, nausea, vaginitis**	**≤4 µg/ml**	

†Factors as defined by Clinical Laboratory Standards Institute (CLSI) [56, 57]

Table 2: Vaccines (under trial) against Methicillin Resistant *Staphylococcus aureus*					
Virulence Factor	**Type (Polyvalent/ monovalent/combined/Pooled)**	**Population targeted**	**Effect (Protective/Not)**	**State (Cinical trial/Rejected/Production)**	**Reason**
Cell surface polysaccharide (Type 5 and Type 8)	**Protein conjugated-bivalent vaccine**	**Dialysis recipients**	**Not protective**	**Clinical trial-Rejected**	**Type 5 and type 8 capsule not present universally**
Poly-N-acetylglucosamine (PNAG)	**Deacetylated PNAG Protein conjugated vaccine**	**-**	**Protective in animal bacteremia model**	**Clinical trial-Undergoing**	**Modest decrease in animal bacteremia model**
Iron surface determinant B (IsdB)	**Protein formulated with amorphous aluminum hydroxyphosphate sulfate adjuvant**	**Cardiac Surgery**	**Protective in animal infection model (Rhesus macaques, mice).**	**Clinical trial-halted**	**Modest protective provided**
Clumping factor A (ClfA)	**High titre anti-ClfA human serum immune preparation Humanized Monoclonal antibody**	**Neonates, Adults with bacterimia**	**Not protective**	**-**	**Phase III neonate trial not successful; Demonstrated efficacy against relapse and complications of bacterimia**
α-hemolysin	**Non toxic α-toxin mutant protein (H35L)**	**-**	**Protective in animal infection model**	**Rejected**	**Does not fully prevent abscess formation.**
Clumping factor B (ClfB)	**Monoclonal antibodies**	**Nasal colonization of S.aureus**	**Protective in animal nasal colonization model (mice)**	**-**	**Reduction in S.aureus colonization in mice was observed when immunized with rClfB.**
IsdA, IsdB, SdrD and SdrE	**Combined vaccine containing purified antigens**	**Universal-targeting wide range of *S.aureus* strains.**	**Protective in animal infection model (mice)**	**-**	**Conferred immune protection in lethal challenge mice model.**
Lipoteichoic acid	**Humanized mouse chimeric mAB**	**Neonates**	**-**	**Clinical trial-undergoing**	**-**
Staphylococcal Protein A (SpA)	**Mutant Protein A (SpA_{KKAA})**	**-**	**Protective in animal infection model (mice)**	**-**	**-**