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EDUCATIONAL GUIDE

MRSA/SA – Emerging Therapeutic & Screening Approaches

BOSCH vivalytic

Introduction

Staphylococcus aureus is a gram positive, commensal bacteria found in normal human flora on the skin and mucous membranes. The commensal nature of this organism results in colonisation of around half of the general population, rising to around 80% in populations of healthcare workers, hospitalised patients and the immunocompromised[1]. However, given the opportunity to colonise internal tissues or the bloodstream, S. *aureus* infection can cause serious disease. Skin conditions caused by S. *aureus* include impetigo, scalded skin syndrome, boils, and abscesses. Examples of more serious conditions include meningitis, pneumonia, endocarditis, bacteraemia, and sepsis[2].

Antimicrobial resistance (AMR) has, and continues to be, one of the largest threats to global health. In 2019, it is estimated that 1.27 million deaths globally were directly attributed to AMR, based on the drug-susceptible counterfactual, with only ischaemic heart disease and stroke accounting for more deaths in that year[1]. Figure 1 shows a global distribution map of MRSA isolates from the data of this comprehensive study. Methicillin-resistant *Staphylococcus aureus* (MRSA) was first identified only one year after the introduction of the penicillin-like antibiotic, methicillin[3]. While methicillin is no longer used in clinical practice, the term MRSA is used to encompass resistance to commercially available antibiotics such as β-lactams[3]. For many years, much work has gone into seeking novel therapies to combat drug-resistant bacteria, however, the indiscriminate overuse of antibiotics seen around the world, along with other factors, continues to contribute to the rise in AMR.

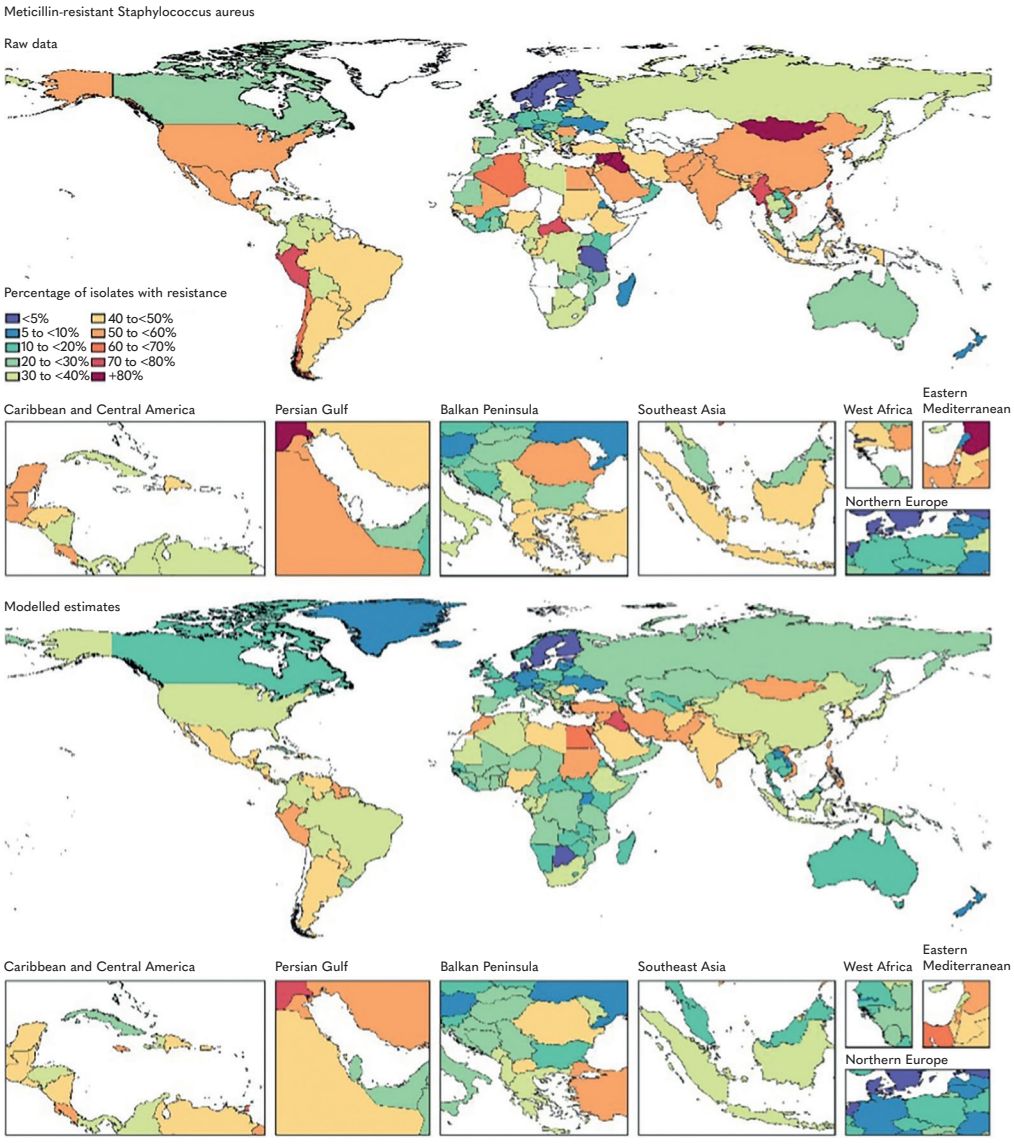

Figure 1. Global distribution of methicillin-resistant isolates of S. aureus in 2019[1]

Identification of drug-resistant strains of bacteria is crucial to allow for characterisation of the pathogen and correct treatment of the infection. Classical evaluation consists of a routine culture to verify a diagnosis based on presenting symptoms. However, this can be a time consuming and laborious process which may delay diagnosis and treatment of a potentially fatal infection[1].

Methicillin-Resistant *Staphylococcus aureus*

Methicillin is of a class of antibiotics known as β-lactams which bind to the penicillin binding protein (PBP) of the bacteria. PBP is responsible for crosslinking between N-acetylmuramic acid and N-acetylglucosamine which forms the architecture of the bacterial cell wall. When β-lactams bind to the PBP, a build-up of peptidoglycan precursors triggers autolytic digestion of peptidoglycan, facilitated by hydrolase. This reduction in peptidoglycans results in the loss of the integrity of the bacterial cell wall and ultimately culminates in cell damage caused by high internal osmotic pressure[3].

While methicillin has lost its clinical utility due to the emergent resistance, MRSA is used to describe S. *aureus* which displays resistance to penicillin-like antibiotics such as amoxicillin and oxacillin, as well as other forms of commercially available antibiotics like macrolides, tetracyclines, and fluroquinolones[4]. A meta-analysis by Dadashi et al., showed that 43% of S. *aureus* isolates where methicillin-resistant, exhibiting the prevalence of MRSA[5].

Transmission is possible from direct contact with an infected individual or through contact with fomites[2]. MRSA infections can be categorised as either community acquired infections (CA-MRSA), or hospital acquired infections (HA-MRSA). While rates of HA-MRSA have fallen over the last ten years, this decrease in infection rates has not translated to CA-MRSA[6]. This is evidence of the requirement for quicker, easier testing in community settings to identify those infected by MRSA and to trigger the initiation of isolation and treatment.

While the pathophysiology of MRSA will largely depend on the causative strain of bacteria, collectively, S. *aureus* is the most common bacterial infection in humans and may result in infections of varying severity including[1]:

- Bacteraemia
- Infective endocarditis
- Skin and soft issue infections
- Osteomyelitis
- Septic arthritis
- Prosthetic device infections
- Pulmonary infections
- Gastroenteritis
- Meningitis
- Toxic shock syndrome
- UTIs

Development of resistance and resistance mechanisms

Antimicrobial resistance arises from a combination of mechanisms. Genetic mutations are crucial in the development of resistance mechanisms. These genetic mutations must favour the survival of the mutated gene and the advantage of AMR mechanisms to the survival of bacteria cannot be understated. Regarding MRSA, S. *aureus* can gain resistance through horizontal gene transfer mediated by plasmids, mutations in chromosomal genes or mobile genetic elements[4]. Methicillin-susceptible *Staphylococcus aureus* (MSSA) gains the staphylococcal cassette chromosome (SCCmec) gene, a gene containing mecA, which is responsible for some of the resistance mechanisms displayed by MRSA[4]. The collection of antibiotics the bacteria gains resistance to, will depend on the SCCmec gene type.

The first mechanism of resistance is the expression of β-lactamase which functions to degrade β-lactams, ultimately resulting in loss of function of the antibiotic. This enzyme hydrolyses β-lactam ions in the periplasmic space, denaturing the antibiotic before it can interact with bacteria[3]. The mecA gene encodes the protein penicillin-binding protein 2a (PBP-2a), a type of PBP which has lower affinity for β-lactams, as well as other penicillin-like antibiotics, due its conformation, meaning that the presence of these antimicrobial agents does not confer a loss of structure in the bacterial cell wall[1].

One study conducted by Hosseini et al., investigated resistance mechanisms in MRSA and showed that all multidrug resistance MRSA strains displayed biofilm formation as part of its resistance strategy[7]. Biofilms induce resistance to high concentrations and a large variety of antimicrobial agents and help regulate anti-bacterial immune responses. Biofilm formation is mediated by the protein, polysaccharide intercellular adhesin (PIA). Furthermore, MRSA strains which display biofilm formation are associated with more severe and more virulent infections[7].

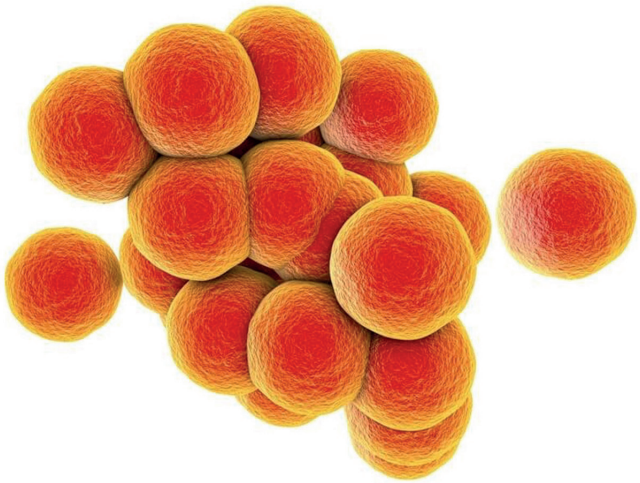

Figure 2. Illustration of Staphylococcus aureus

Current and Emerging Therapeutic Strategies

Other types of antibiotics have been used to treat MRSA infections over the years. Vancomycin has been used to combat infections resistant to penicillin-like antibiotics as they display a different mode of action. Vancomycin inhibits peptidoglycan synthesis by forming hydrogen bonds within the structure of peptidoglycan precursors[2]. While this strategy has proven effective for past 50 years, more and more strains are displaying vancomycin resistance in addition to resistance to penicillin-like antibiotics[8]. One study by Deyno et al., estimates the prevalence of vancomycin-resistant S. *aureus* in Ethiopia to be around 11%[4]. Daptomycin is another antibiotic which has been shown to be effective in MRSA treatment. This cyclic lipopeptide binds to the bacterial membrane, resulting in cell death[9].

Due to the decreasing number of available, effective antibiotics, novel therapeutic strategies are required to combat MRSA infection. One of the most promising approaches uses antimicrobial peptides (AMPs). AMPs are naturally occurring molecules of the innate immune system and have one of two mechanisms of action: membranolytic action and non-membranolytic action. AMPs normally consist of and amphipathic or cationic structure, between 5-50 amino acids long. Naturally occurring AMPs have been used as a model to develop synthetic AMPs, designed to neutralise the limitations of natural AMPs boasting an improved half-life and improved antimicrobial properties[3].

Non-membrane disruptive AMPs require much more investigation; however, it is accepted that these AMPs enter the cell, reacting with important intracellular components inhibiting protein and nucleic acid synthesis, cell division and protease activity[3].

Silver nanoparticles (AgNPs) exhibit broad spectrum antimicrobial properties through various mechanisms of action. These nanosized particles boast increased antimicrobial properties due to an increased surface area per volume ratio. The first mechanism of action to note is AgNPs direct adhesion to the bacterial membrane, which alters the structural integrity of the membrane, allowing the AgNPs to penetrate the cell, wreaking havoc on the intracellular components until it loses the ability to carry out essential cellular processes[3].

Once the AgNPs aggregate on the bacterial surface, the difference in electrostatic charge, driven by the positive charge displayed by the AgNPs and negatively charged bacteria, causes pit formation to occur on the cell surface, inhibiting vital cellular movement, resulting in cell death[3]. AgNPs may also inhibit protein synthesis by denaturing ribosomes and directly interacting with DNA. This interaction can cause denaturing of the DNA helix and ultimately result in cell death[3]. Finally, AgNPs can induce the production of reactive oxygen species (ROS) and free radicals. The molecules cause irreversible cell damage to the bacteria[3].

While AMPs and AgNPs each possess individual limitations such as toxicity and instability, studies show that a combination of these therapeutic strategies can overcome these issues, stabilising the antimicrobial agents to their respective target sites[3].

Screening, Testing & Evaluation

Classical determination of MRSA and other bacterial infections consists of obtaining a patient sample and growing colonies from the patient sample in culture. These cultures can then be investigated under a microscope and characterised, allowing diagnosis and the initiation of treatment. Whilst effective, these methods are time consuming and laborious, taking up to three days for cultures to develop, somewhat limiting their utility for the diagnosis of potentially fatal infections.

New molecular rapid PCR microbiology techniques aid in the identification of bacterial strains through a three-step process involving extraction, amplification, and detection. These new methods allow for timely identification of infectious strains and AMR characterisation. Specific genes or sections of gene which are responsible for AMR can be detected, helping to achieve strain characterisation and aid physicians in prescribing the correct treatment plan. These methods improve test turnaround times to around one to two days and help to reduce the risk of costly human error and contamination.

Figure 3. Staphylococcus aureus culture

Vivalytic MRSA/SA

Bosch Vivalytic MRSA/SA is an automated, qualitative, in vitro diagnostic test based on real-time PCR for the detection and differentiation of Methicillin-resistant *Staphylococcus aureus* (MRSA) and Methicillin-sensitive *Staphylococcus aureus* (MSSA) DNA from human nasal- or oropharyngeal swabs to aid in the diagnosis of MRSA infection of symptomatic or asymptomatic individuals, providing results in less than 1 hour.

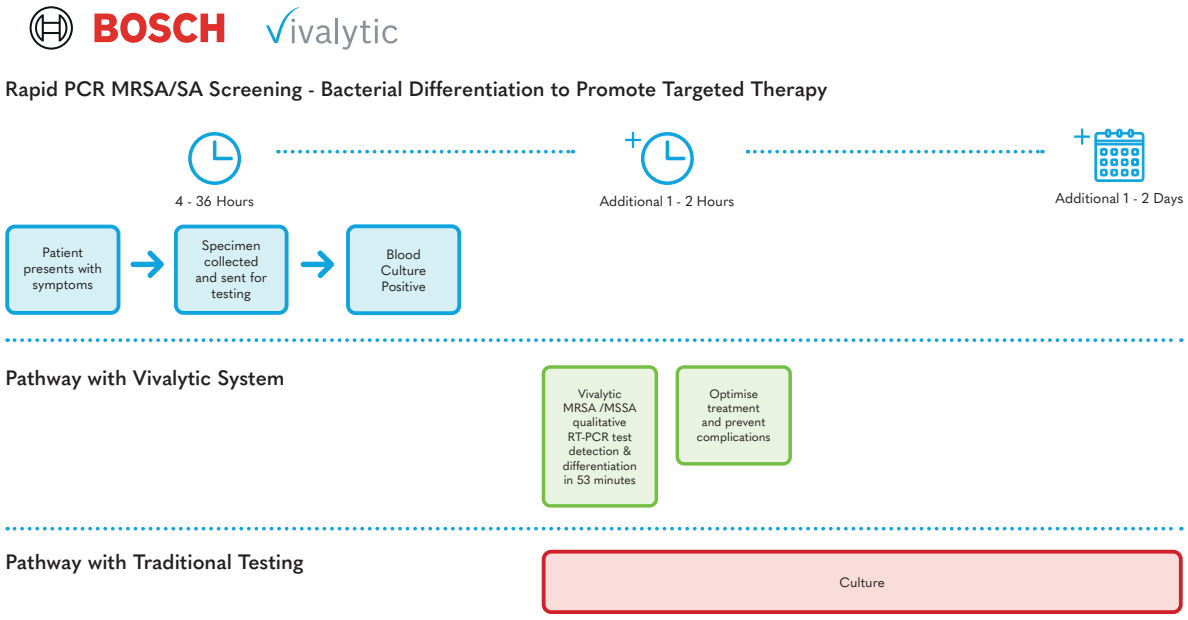

Figure 4. Vivalytic vs traditional culture methods

Without MRSA screening, many MRSA colonised patients remain unnoticed in hospitals and will not be isolated. Without Isolation many of these patients transfer the pathogen to at least one other patient during their hospital admission. PCR based screening is associated with high precision and fast time to results and is often used for early decisions on isolation and hygiene measures.

This POCT system provides fast, accurate characterisation of MRSA/SA strains while minimising the required user steps and reducing the need for expensive laboratory equipment helping physicians implement timely and effective treatments. The table below details the benefits of this array:

Pathogen	Description
Detectable Pathogens	Methicillin-resistant *Staphylococcus aureus* Methicillin-sensitive *Staphylococcus aureus*
Specific Gene Targets	SCCmec/orfX junction MecA/MecC SA422
Multiple Sample Types	Data shows that for approx. 13% of MRSA carriers, the pathogen is only located in the throat. Therefore, using throat swabs significantly increases the sensitivity of detection by approx. 26%.
Broad MRSA Range	mecA or mecC are the genes responsible for resistance to β-lactam antibiotics. mecA/meC is part of the mobile genetic element Staphylococcal cassette chromosome mec (SCCmec). Vivalytic MRSA/SA can detect mecA as well as mecC and a broad variety of SCCmec elements which help to reduce false negative results.
Fast time-to-result	Provides quick results in less than 1hr allowing quick decisions on therapies. Traditional culture time-to-result is 48-72hrs and laboratory PCR is 12-24hrs.
Highly Automated	This highly automated system minimises the user steps required to achieve a result while limiting the requirement for expensive lab equipment and sample transportation. Vivalytic MRSA/SA POCT test allow the implementation of treatment as soon as 1hr after sample collection.

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