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Brief Insight about Methicillin-Resistant Staphylococcus aureus

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Abstract: Background: Methicillin-resistant Staphylococcus aureus (MRSA) represents a significant threat to global public health. This bacterium, initially emerging in hospitals shortly after the introduction of methicillin, has become widespread in both healthcare and community settings. Its resistance to methicillin, and subsequently many other beta-lactam antibiotics, severely limits treatment options and contributes to increased morbidity, mortality, and healthcare costs. MRSA infections manifest in a variety of ways, ranging from relatively minor skin and soft tissue infections (SSTIs) like boils and abscesses, to more serious invasive infections such as pneumonia, endocarditis, and sepsis. The acquisition of the mecA gene, carried on a mobile genetic element called the staphylococcal cassette chromosome mec (SCCmec), is the primary mechanism conferring methicillin resistance. This gene encodes penicillin-binding protein 2a (PBP2a), which has a low affinity for beta-lactam antibiotics, enabling cell wall synthesis to continue even in their presence. Various SCCmec types exist, each with different genetic characteristics and associated with specific MRSA clones. The spread of MRSA is facilitated by its ability to colonize both nares and skin, allowing for transmission through direct contact, contaminated surfaces, and airborne particles. Effective infection control measures, including hand hygiene, appropriate disinfection protocols, and judicious antibiotic use, are crucial for limiting MRSA transmission. Ongoing research focuses on developing new antibiotics, alternative therapies such as bacteriophage therapy, and vaccines to combat this persistent and evolving pathogen. Understanding the epidemiology, resistance mechanisms, and virulence factors of MRSA is essential for developing effective strategies for prevention and treatment.

Keywords: *Methicillin-Resistant, Staphylococcus aureus*

Introduction

Bacteria of the genus Staphylococcus are gram-positive cocci irregular, grapelike clusters. The term Staphylococcus is derived from the Greek term staphyle, meaning a bunch of grapes {1}.

Staphylococci are non-motile, non spore forming and catalase positive bacteria. The cell wall contains peptidoglycan and teichoic acid. The organism is resistant to temperatures as high as 50□C, to high salt concentration and to drying. Colonies are usually large, smooth and translucent, most strains are pigmented ranging from yellow to orange {2}.

S. aureus is found in the environment and is also found in normal human flora, located on the skin and mucous membranes (most often the nasal area) of most healthy individuals {3}.

S. aureus does not normally cause infection on healthy skin; however, if it is allowed to enter the bloodstream or internal tissues, these bacteria may cause a variety of potentially serious infections {4}.

Staphylococcus aureus is a successful pathogen due to a combination of numerous bacteria immune-evasive strategies. It is a facultative anaerobic and has been recognized as a major pathogen of hospital acquired infection and treatment of staphylococcal infections is complicated by the ability of this bacterial species to become resistant to antibiotics {5}.

Pathophysiology:

Staphylococcus aureus is a microbe carried by humans. It can cause minor, severe, or fatal infections under particular circumstances, such as minor skin and soft-tissue infections, food poisoning, and life-threatening diseases like bacteremia and infective endocarditis {6}.

It is one of the five most common causes of hospital-acquired infections and one of the primary causes of wound infections following endocarditis surgery {7}.

The organism may cause disease through tissue invasion and toxin production. The toxins liberated by the organism may have effects at sites distant from the focus of infection or colonization {8}.

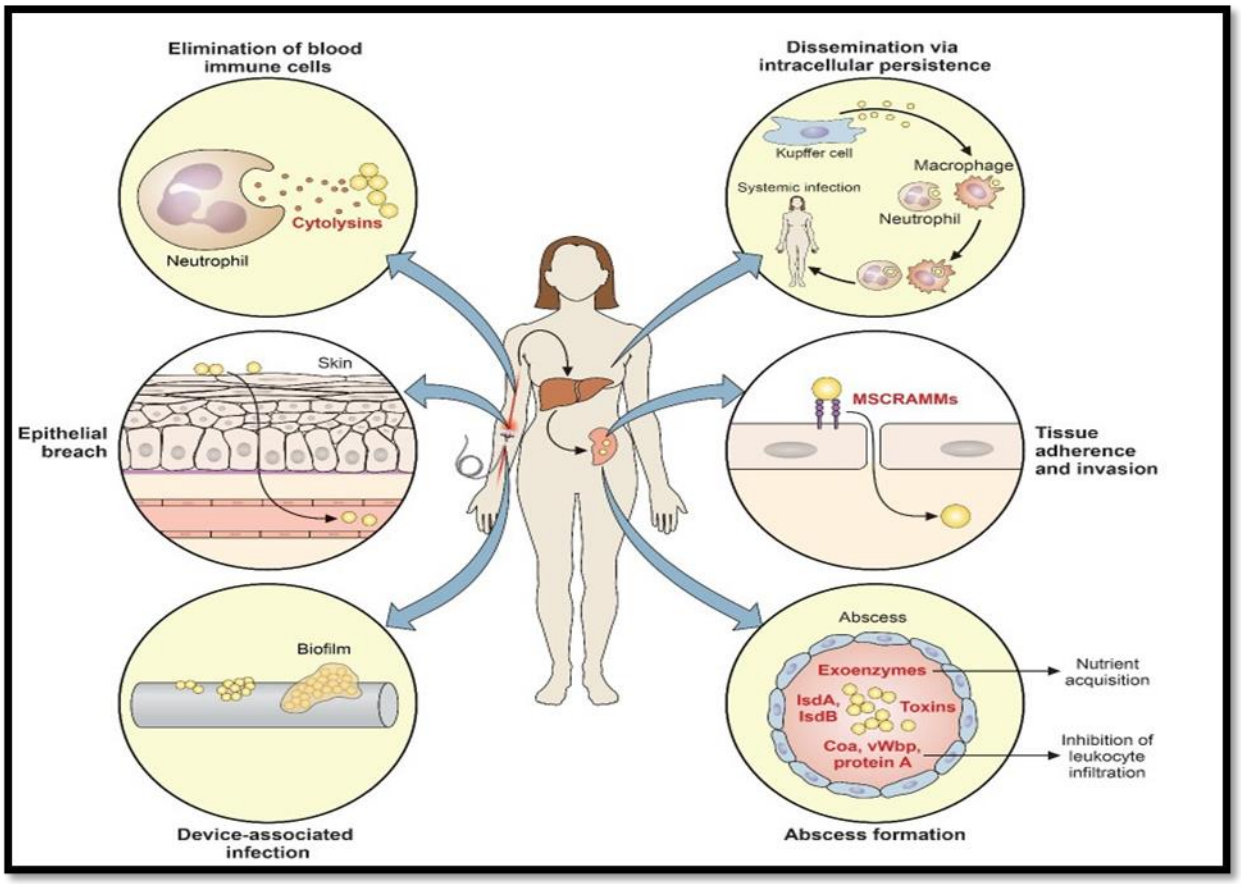
Tissue invasion:

The postulated sequence of events that leads to infection is initiated with carriage of the organism. The organism is then disseminated via hand carriage to body sites where infection may occur (either through overt breaks in dermal surfaces, such as vascular catheterization or operative incisions, or through less evident breakdown in barrier function, such as eczema or shaving-associated microtrauma {9}.

The hallmark of staphylococcal infection is the abscess, which consists of a fibrin wall surrounded by inflamed tissues enclosing a central core of pus containing organisms and leukocytes. From this focus of infection, the organisms may be disseminated hematogenously, even from the smallest abscess. The ability to elaborate proteolytic enzymes facilitates the process. This may result in pneumonia, bone and joint infection, and infection of the heart valves. In immunocompromised hosts (eg, patients with cancer who are neutropenic and have a central venous line), 20-30% develop serious complications or fatal sepsis following catheter-related S. aureus bacteremia.

Persistent deep-seated infections have now been linked to small-colony variants of the organism. This population is more resistant to antibiotics and grows slowly. These organisms have been described in patients with cystic fibrosis and may contribute to the persistence of S. aureus in these patients {10}.

Staphy aureus systemic infection frequently begins with a breach of the bacteria through the protective barrier of the skin or dissemination froma biofilm that can form on indwelling medical devices. In the bloodstream, the bacteria can actively attack and eliminate immune cells suchas neutrophils via cytolytic toxins, or – alternatively – persist in such cells to achieve systemic distribution. Passage through the liver, wherethe bacteria are confronted by the phagocytic activity of Kupffer cells, represents a bottleneck for subsequent systemic infection. If the bacteria survive this stage, they can further distribute through the blood stream and attach to and invade tissue cells, which is mediated by MSCRAMM surface proteins. Subsequent abscess formation is impacted by many different bacterial factors that include specific surfaceproteins, toxins, and exoenzymes. {11}.



Figure(1): Stages of S. aureus systemic infection. Toxin-mediated disease:

The organism also elaborates toxins that can cause specific diseases or syndromes and likely participate in the pathogenesis of staphylococcal infection. Enterotoxin-producing strains of S. aureus cause one of the most common food-borne illnesses {10}.

A rare but well-described disorder in neonates and young children is staphylococcal scaled skin syndrome (Ritter disease). The organism produces an exfoliative toxin produced by strains belonging to phage group II. Initial features include fever, erythema, and blisters, which eventually rupture and leave a red base. Gentle shearing forces on intact skin cause the upper epidermis to slip at a plane of cleavage in the skin, which is known as the Nikolsky sign. How the exfoliative toxins produce epidermal splitting has not been fully elucidated {12}.

The most feared manifestation of S. aureus toxin production is toxic shock syndrome (TSS). Although first described in children, it was most frequently associated with women using tampons during menstruation. These toxins are superantigens, T-cell mitogens that bind directly to invariant regions of major histocompatibility complex class II molecules, causing an expansion of clonal T cells, followed by a massive release of cytokines. This cytokine release mediates the TSS; the resultant pathophysiology mimics that of endotoxic shock {13}.

Staphylococcus aureus Infection Clinical Presentation

Skin and soft tissue infection (impetigo) is a small area of erythema that progresses into bullae (filled with cloudy fluid) that rupture and heal with the formation of a honey-colored crust. Although group A Streptococcus was once considered the primary agent, Staphylococcus aureus has become the major pathogen since the 1980s {14}.

Scalded skin syndrome (Ritter disease):

An exfoliative toxin causes this relatively rare syndrome, which takes the form of superficial fragile blisters that burst, leaving a tender base. The patient is often febrile and, occasionally, has mucopurulent eye discharge {15}.

Folliculitis, furuncle, and carbuncle:
These are increasingly severe staphylococcal skin infections. Folliculitis is a tender pustule that involves the hair follicle. A furuncle involves both the skin and the subcutaneous tissues in areas with hair follicles, such as the neck, axillae, and buttocks. They are actually small abscesses characterized by exuding purulent material from a single opening {16}.
Bone infections (osteomyelitis):
Children often present with sudden onset of fever and bony tenderness on the limb. The pain may be throbbing and severe; however, presentation in neonates can be subtle. Infants may appear well, except for failure to move extremily or pain on movement {16}.
Septic arthritis:
Typical findings include decreased range of motion, warmth, erythema, and tenderness of the joint with constitutional symptoms and fever. Infants (in whom the hip is the most commonly involved joint) are an exception, as these signs may be absent {17}.

Endocarditis:
The initial presentation of patients with S aureus endocarditis is fever and malaise. However, the disease has a more rapid onset than that caused by less virulent pathogens {18}.

Toxic shock syndrome (TSS):
Severe myalgia or elevated creatine kinase (CK) levels are observed. Myalgia may be one of the earliest manifestations of the disease.
Hepatic levels of bilirubin, serum glutamic-oxaloacetic transaminase (aspartate aminotransferase), and serum glutamic-pyruvic transaminase (alanine aminotransferase) are twice the upper limit of the reference range {19}.
Pneumonia
Cases of rapidly progressive and fatal staphylococcal pneumonia still occur, although they were much more common in the 1950s and early 1960s, when S aureus phage type 80/81 caused frequent disease in infants. Staphylococcal pneumonia most commonly occurs in infants, young children, and patients who are debilitated. This is a rapidly progressive disease. Patients with primary staphylococcal pneumonia present with a short prodrome of fever followed by rapid onset of respiratory distress, which may include tachypnea, retractions, and cyanosis {20}.
Thrombophlebitis
Usually occurring in a hospitalized patient, thrombophlebitis is characterized by fever, pain, and, occasionally, erythema at the insertion site of an intravenous catheter. Occasionally, pus is expressed with severe suppurative thrombophlebitis in burn patients than alive ones {21}.
Deep tissue abscess and infection:
Muscles (myositis and pyomyositis) and organs can become infected, including the parotid gland, eyes, liver, spleen, kidneys, and central nervous system. Deep abscesses also may occur. These infections typically cause fever with or without localizing pain {22}.
Diagnosis of Staphylococcus aureus infection:
Both community-associated and hospital acquired infections with Staphylococcus aureus have increased in the past 20 years and the rise in incidence has been accompanied by increase in antibiotic-resistant-stress {23}.
Diagnosis of Staphylococcus infection depend on types and presentation of S. aureus infection including the following {23}.

Folliculitis, Furuncle and Carbuncle.
• Diagnosis based on clinical appearance.
• Aspiration or incision and culture of purulent material from the lesion occasionally diagnostic.
Osteomyelitis:
• Culture of bone aspirate.
• Blood culture, results positive in only 30-50% of pediatric patients.
• C-reactive protein levels and erythrocyte sedimentation rate generally elevated in acute disease.
• Bone scan with increased technetium-99m-labelled diphosphonate uptake supports the clinical diagnosis however, the modality is not as useful in neonates or after trauma or surgery.
• MRI is the best imaging modality for defining purulent collections and for planning surgery.
• On plain film radiographs, destructive bone changes one usually observed 2 weeks after infection {13}.
Septic arthritis:
• Gram stain and culture of Joint fluid is the primary means of diagnosis.
• Direct inoculation of synovial fluid into culture bottlers may improve culture yield.
• Median while blood cell count in joint fluid is 60.5x109 with neutrophil predominance >75%.
• Synovial fluid glucose levels is often low.
• Plain radiographs show capsular swelling.
• MRI or CT scanning is the imaging method of choice for pyogenic sacroilitis {24}.
Endocarditis:
• Blood culture is the most important diagnostic procedure.
• Inject the blood sample into hypertonic media if the patient has been exposed to antibiotics.
• Obtain 3-5 sets of large-volume blood cultures with the first 24 hours.
• Echocardiography is a valuable adjunct.
{25}
Pneumonia
• Blood culture are more likely to be positive in secondary than primary disease (90% vs 20%).
• An adequate respiratory tract specimen should be obtained prior to initiating therapy, specimens may include endotracheal sampling, pleural fluid or lung tap.
• Sputum specimens are inadequate because upper respiratory tract colonization is common.
• No radiologic features are highly specific.
• Typical radiographic features are unilateral consildation in primary Staphyloccal pneumonia and bilateral infiltrates in secondary cases.
• Early in the disease course. The chest radiograph may reveal minimal infiltrates, but within hours infiltrates progress rapidly.
• Pleural effusion, pneumatoceles and pneumothorax are also common.
• On oncology patients, S. aureus may cause pulmonary nodules {26}.

Thrombophlebitis:
• Obtain blood culture through the intravenous line and peripheral blood culture {23}.
Conventional approaches for Staphylococcus aureus identification:

Tube coagulase tests or a latex agglutination test should be used for routine identification of staph. aureus.

Identification using DNases tests and negative results in slide coagulase tests should be confirmed with a tube coagulase or latex agglutination test.

Susceptability testing by:

a- A standard recognized method {27}.
b- Latex method detection pencillin binding protein 2a (PBP2a) and/or polymerase chain reaction (PCR) methods detecting the mecA gene may be used for confirmation of equivocal results {28}.
Management of Staphylococcus infection:
Antibiotic regimens include the following:
• Emperic therapy with pencilin or cephalosporins may be inadequate because of community methicillin-resistant Staphylococcus aureus (CA-MRSA) {29}.
• Combination therapy with penicillinase-resistant pencillin of cephalosporin and clindamyain or quinolone {30}.
• Clindamycin, trimethoprin-sulfamethoxazole (TMP-SMX), rifampin, doxycycline or quinolone.
• TMP-SMX and rifampin in combination rather than singly.
• Clindamycin may become the preferred outpatient therapy in regions with relatively low incidence of clindamycin resistance {31}.

Methicillin-Resistant Staphylococcus aureus
MRSA or methicillin resistant Staphylococcus aureus is an especially virulent form of bacteria capable of producing severe, potentially lethal infections. Due to the seriousness of MRSA-related illness, rapid detection is crucial. Early identification and prompt treatment usually offers a more favorable outcome {9}.
MRSA is any strain of Staphylococcus aureus that has developed, through the process of natural selection, resistance to beta lactam antibiotics {9}.
Strains unable to resist these antibiotics are classified as methicillin sensitive Staphylococcus aureus or MSSA. The evolution of such resistance does not cause the organism to be more intrinsically virulent than strains of S. aureus that have no antibiotic resistance, but resistance does make MRSA infection more difficult to treat with standard types of antibiotics and this more dangerous {32}.
MRSA probably arose by succession of mutation and acquisition of the resistance plasmids. The resistance gene mecA and regulatory sequences that encode for production of low-affinity pencillin-binding protein (PBP-2a) are not present in methicillin-sensitive strains {33}.
The importance of MRSA strains is that in addition of being resistant to methicillin, most strains are also resistant to the □-lactam antibiotics with the exception of glycopeptides antibiotics {34}.
The increased incidence of MRSA has led to more frequent use of vancomycin, the drug commonly relied on for treating MRSA infections {35}.
MecA encodes a protein PBP2A, a peptidoglycan transpeptidase with extremely law affinity for the entire large family of □-lactam antibiotics {36}.
The presence of this protein plays a critical role in allowing MRSA strains to continue synthesis of peptidoglycan and bacterial growth in the presence of high concentrations of □-lactam antibiotics {37}.
In contrast to the common molecular mechanism of resistance, individual MRSA clinical isolates differ widely in their susceptibility to □-lactam antibiotics with individual MRSA strains presenting methicillin MIC values as low as a few □g/ml up to several hundered □g/ml depending on the particular MRSA clone, and this variation in resistance level cannot be explained by transcriptional regulation of mecA through the activity of regulatory elements such as the mec1 and mecR1 or bla1 and blaR1 genes {38}.
MRSA is usually acquired during exposure to hospitals and other healthcare facilities and causes a variety of serious healthcare-associated infections. The problem is exacerbated by the propensity of the organism to cause cross-infection and its ability to colonize individuals for months or years {39}.
Mechanism of Methicillin Resistance
Methicillin resistance is defined as the strains of S. aureus that are resistant to the isoxazoyl penicillins such as methicillin, oxacillin and flucloxacillin. MRSA are cross-resistant to all currently licensed □-lactam antibiotics.

The expression of methicillin resistance by S. aureus strains is by virtue of acquired penicillin binding proteinPBP2a, encoded by mec A gene. Structurally, PBP2a possesses both transglycosylase and transpeptidase. PBP2a confer resistance to all ?-lactam antibiotics. The origin of mec A gene is unknown. Expression of methicillin resistance in S. aureus is commonly under regulatory control by mec I or by Bla I gene. The mec I and bla I repressors are controlled by the mec RI and bla RI transducers. Expression of methicillin resistance in S. aureus is also influenced by the expression of other genetic loci called fem ("factors essential for methicillin resistance") or aux ("auxiliary") genes {34}. Many fem and aux factors have now been identified, which are involved in formation of the staphylococcal cell wall. The mec A gene is located within a larger region of chromosome known as the staphylococcal cassette chromosome mec (SCCmec) region (21-67 kb) {40}. SCCmec is a mobile element, with mobility conferred by the presence of the ccrA and ccrB genes. The basic elements of SCCmec are the mecRI-mecI-pbp2a region and ccrA. Nosocomial isolates have larger SCCmec, owing to the accumulation over time of integrated plasmids or transposons that contribute to the multi-drug resistance {40}. There are five currently described SCCmec types (types I, II, III, IVa, IVb, V). Types I, II and III are found predominantly in healthcare-associated MRSA, whereas type IV is commonly found in the more susceptible community-associated MRSA {41}. Type IV SCCmec element is small and transferable by transduction. Types I to III SCCmec elements are large and hence do not transfer by bacteriophage. They are predominantly transferred by person-to-person spread of MRSA in the hospital rather than the spread of the resistant determinant from strain to strain. The spread of MRSA within institutions is therefore largely due to the transmission of resistant organisms from patient to patient, probably on the hands of transiently colonized healthcare workers {42}.

Once MRSA is introduced into a healthcare setting, transmission and persistence of the resistant strain is determined by the availability of vulnerable patients, selective pressure exerted by antimicrobial use, increased potential for transmission from larger numbers of colonized or infected patients ("colonization pressure"), and the impact of implementation and adherence to prevention efforts {43}. Patients vulnerable to colonization and infection include those with severe disease, especially those with compromised host defenses from underlying medical conditions; recent surgery; or indwelling medical devices (e.g., urinary catheters or endotracheal tubes) {44}. Hospitalized patients, especially ICU patients, tend to have more risk factors than nonhospitalized patients and have the highest infection rates. Studies have shown high prevalence (7%) of MRSA colonization at the time of patient admission {9}. MRSA acquisition has been shown to occur both in the healthcare setting and in the community {45}. A significant number of patients (2.2% of all adults admitted to the hospital) are colonized with community acquired CA-MRSA USA300 clone at the time of admission, which represents an emerging and increasingly problematic reservoir of MRSA {9}.

The emergence of new epidemic strains of MRSA in the community, among patients without established MRSA risk factors, may present new challenges to MRSA control in healthcare settings. Community stains of MRSA (e.g., USA300 and USA400) are being reported with increasing frequency within hospitals {46}.

Changing resistance patterns of MRSA in ICUs in the National Nosocomial Infection Surveillance (NNIS) system from 1992 to 2003 provide additional evidence that the new epidemic MRSA strains are becoming established as healthcare-associated or community pathogens {47}.

Infections with these strains have most commonly presented as skin disease in community settings. However, intrinsic virulence characteristics of the organisms can result in clinical manifestations similar to or potentially more severe than traditional healthcare-associated MRSA infections among hospitalized patients. The prevalence of MRSA colonization and infection in the surrounding community may therefore affect the selection of strategies for MRSA control in healthcare settings {9}.

Global Epidemiology of MRSA

Bacterial strain typing distinguishes epidemiologically related or clonal isolates from unrelated isolates. Epidemiologically related isolates are viewed as descendants from a common precursor cell.

Numerous techniques are available to differentiate S. aureus, and specifically MRSA, isolates. Historically, isolates were distinguished by phenotypic methods, including antibiotic susceptibility testing and bacteriophage typing. Both methods have limitations, as genetically unrelated isolates commonly have the same antibiogram, and many S. aureus isolates are nontypable by phage typing {48}.

With the advance of molecular biology, strain typing is focused on DNA-based methods. Initial techniques compared restriction endonuclease patterns of chromosomal or plasmidDNA. The second-generation of genotyping methods included s southern blot hybridization using gene-specific probes, ribotyping, polymerase chain reaction (PCR)-based approaches, and pulsed-field gel electrophoresis (PFGE) {49}. These methods require subjective interpretation and comparison of patterns and fingerprint images. In addition, image-based methods do not provide biological criteria to evaluate the relatedness between different strains {50}.

DNA sequence analysis is an objective genotyping method; the genetic code (A-T-C-G) is highly portable and easily stored and analyzed in a relational database. Recent advances in DNA-sequencing technology, including rapid, affordable, high-throughput systems, have made it possible for sequencing to be considered as a viable typing method. Two different strategies have been used to provide genotyping data: multilocus sequence typing (MLST), which compares sequence variation in numerous housekeeping gene targets; and single-locus sequence typing, which compares sequence variation of a single target among strains to be typed {9}.

Two S. aureus genes conserved within the species, protein A (spa) and coagulase (coa), have variable short-sequence repeat (SSR) regions constructed from closely related 24- and 81-bp tandem repeat units, respectively. In both genes, the in-frame SSR units are degenerative, variable in number and variable in the order in which repeat units are organized. The genetic alterations in SSR regions include both point mutations and intragenic recombination that arise by slipped-strand mispairing during chromosomal replication and that result in a high degree of polymorphism {51}.

Synergistic activity of MRSA in hospitals.

When patients with MRSA have been compared to patients with methicillin-susceptible S. aureus (MSSA), MRSA-colonized patients more frequently develop symptomatic infections {52}. Furthermore, higher case fatality rates have been observed for certain MRSA infections, including bacteremia, post-sternotomy mediastinitis and surgical-site infections {53, 54}. Also some studies have reported an association between MRSA infections and increased length of stay, as well as healthcare costs, while others have not {55}.

It was reported that a majority of MRSA strains were from wound infections (56.9%), with pneumonia cases being the second most common (21.0%) followed by blood stream infection (15.1%) and urinary tract infection (6.9%) being the third & the fourth most common cases respectively {56}.

The most of striking situation is that MRSA strains have emerged with concomitant resistance to many commonly used antibiotic of groups aminoglycosides, macrolides, fluroquinotones, chromaphenicol and tetracycline {57}.

In addition, MRSA isolates are often found resistant to cephalosporins, cefems and other □-lactams, ampicillin-sulbactam, amoxicillin-clavanic acid, tiearcillin-clavalanic acid, piperacillin-tazobactam and carbapenem imipenem. Thus MRSA isolates are multidrug resistant (MDR). This MDR-MRSA is a new or rather a continually evolving paradigmatic pathogen {58}.

Indiscriminute uses of antibiotics could be regarded as the cause of emergence of MDR-MRSA as in regions where the availability of antibiotics is limited, further-more nosocomial infections are expected from intensive care units because of the severity of infection by one or more pathogens in patients with wounds {59}.

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