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Review Article

Methicillin-Resistant *Staphylococcus aurous:* Epidemiology, Transmission and New Alternative Therapies: A Narrative Review

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Abstract

Over the last decade, we were facing medical struggle by the emergence of multi-resistant bacteria, especially methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA infections are still causing a growing global concern due to the rapid adaptive multidrug resistance to conventional antibiotics in human, community and veterinary medicine. Here we provide an overview about MRSA epidemiology, transmission and alternative potential treatments particularly new discovered phytochemicals with biological activity. In this narrative review, bibliographic data was collected from literature search databases: Google Scholar, web of science and PubMed/MEDLINE during recent years (2016 to 2021). MRSA is responsible of wide spectrum life threatening infections such us septicemia, endocarditis, and wound infections. It has epidemic potential in hospitals, that is responsible of most nosocomial infections leading to mortality and constitute a real burden for the healthcare systems. Effective preventive strategies for management of MRSA are highly required moreover; the identification and development of novel drugs or active biomolecules through phytochemicals are time challenging to face new resistant strains.

Keywords: Methicillin-resistant *Staphylococcus aureus* (MRSA); Alternative treatments

Introduction

Staphylococcus aureus was and still a pathogen of great concern clinically and in community because of its high virulence and its ability to cause a wide spectrum life threatening infection (1).

S. aureus is a genus of gram-positive spherical bacteria (coccal bacteria) that commonly cause infectious diseases in human, such as skin infections, infective endocarditis, osteomyelitis, septic arthritis and metastatic abscess formation, respiratory disease, and food poisoning (1). *S. aureus* can colonize the skin and the mucous of the respiratory tract of both human and animals. The bacterial colonization is asymptomatic but increases the risk of secondary infections such as superficial skin lesions and soft tissue infections, and sepsis (2).

Many studies have been conducted on *S. aureus*, in order to better understand the pathogenic mechanism, in addition to therapeutic researches attempts on staphylococcal vaccine, have been

designed to effectively combat these bacteria but infections are still increasing (1-3).

Treatment for *S. aureus* infections depends on the type of infection, but antibiotics are commonly prescribed such as penicillin and its derivatives, including methicillin (4). However, some *S. aureus* infections no longer respond to common antibiotics and remain a major threat that is difficult to manage due to the development of large and diverse *S. aureus* strains resistant to broad spectrum of antibiotics, which results from misuse and or overuse of antibiotics.

S. aureus strains that have developed or acquired a multidrug-resistance, are commonly known as methicillin resistant *S. aureus* (MRSA) (5).

The diversity and wide dissemination of new MRSA strains, in the past few decades, highlights the need for a better understanding of the epidemiology and transmission of MRSA, for a good management and effective prevention strategies. These infections cause a global concern that represent a major cause of nosocomial or hospital-associated (HA-MRSA) infections. Moreover, MRSA strains can invade community settings and infect healthy people without predisposing risk factors leading to Community-Acquired MRSA (CA-MRSA) infections. These infections can also expand to livestock that generate Livestock-Associated MRSA (LA-MRSA).

Therefore, we provide an overview of the current clinical MRSA knowledge, epidemiology of MRSA in hospitals, community settings and livestock, source of transmission and new alternative therapies.

MRSA

Since the discovery of the bactericidal effects of penicillin in 1929 by Alexander Fleming, penicillin was widely used to treat bacterial infections during World War II which resulted in declining death rates from bacterial pneumonia and meningitis around the world (6).

Subsequently, penicillin becomes a major driver for *S. aureus* resistance selection.

The use of penicillin in 1940s, considerably improved the prognosis of patients with *S. aureus* infections. However, soon after, the extensive use has led to the emergence of penicillin resistance, first detected in hospitals and then in community caused a global concern (7, 8).

Penicillin-resistant strains of *S. Aureus* acquired resistance to all β-lactam antibiotics due to the production of enzyme called β-lactamase "penicillinase". This enzyme hydrolyzes the antibacterial agent and makes it ineffective (7).

In 1959, chemists have introduced the first semi synthetic penicillinase-resistant penicillins, known as methicillin in order to combat β-lactamase hydrolysis caused by gram-positive bacteria (*S. aureus*). Methicillin consists in a structural change in penicillin that confers resistance to penicillinase by inhibiting the bacterial cell wall synthesis.

However, in 1961 and little after their first clinical administration, methicillin-resistant strains of *S. aureus* emerged. The first MRSA strain was observed in a hospital in the United Kingdom. Soon after, strains were found in other healthcare facilities in many other countries. They become then, a worldwide problem in hospitals and in the community due to the emergence of resistance to several antibiotic classes that limit treatment options which is responsible for morbidity and mortality (7, 6).

Initially, MRSA infections are of human origin until in 1972 they were isolated from cow mastitis.

Thereafter, MRSA infections have also been identified in domestic, wild animals and terrestrial / aquatic species (9). Livestock have been also identified as an important reservoir of livestock-associated MRSA (LA-MRSA) and responsible for zoonotic transmission to humans via food chains, and/or via animal wastes (9).

The misuse and overuse of antimicrobials in human therapy, in animals, in agriculture and in aquaculture has led to the development of antimicrobial resistance, which is a severe global concern that threats not only human health but also overlap to animals and environmental health. This resistance has social and economic negative impact such as the increase of mortality in humans and animals (10).

Genetics of MRSA

Molecular researches suggest that unlike penicillin resistance gene located on a plasmid, the MRSA resistance to beta-lactam antibiotics is due to *mec* genes (*mecA* and its homolog *mecC*) located on a mobile genetic element known as the staphylococcal cassette chromosome mec (SCCmec). The *mec A* gene encodes a penicillin-binding protein 2a (PBP2a) that plays a key role in bacterial cell wall synthesis (9). The PBP2a has a decreased binding affinity for most semisynthetic penicillin, so that the activity of cell wall peptidoglycan synthesis continue in MRSA strains despite the presence of several β-lactam antibiotics (11).

Methicillin resistance *S. aureus* strains can also be mediated by the *mecC* gene, initially termed $mecA_{LGA251}$ is a homologous of *mecA* that shares only 69% nucleotide sequence homology. The MRSA mecC isolates was identified not only in human but also in animals in many European countries (12). Although *mecC* gene also encode PBP2a and plays key role in determining β-lactam resistance, researches emphasized significant dissimilarities in proteins properties. In fact, both proteins showed different antibiotic resistance profile: thePBP2a mecC showed a higher affinity for oxacillin than for cefoxitin, while PBP2a mecA showed a higher resistance to cefoxitin than oxacillin (13).

Epidemiology of MRSA

The MRSA typing methods aimed at characterizing and determining the extent of the spread of strains, have allowed the identification of different lineages which are epidemic to different regions around the globe. These lineages can be zoonotic, human associated and/or host specific. The first epidemic MRSA (EMRSA) infection was recognized early in 1980s in the United Kingdom and then the spread increased all over the world. Overall, 17 EMRSA strains have been described but the most predominant strains are EMRSA-15 (ST22) and EMRSA-16 EMRSA-16 (ST36) and became predominant HA-MRSA strains (14).

The majority of isolates belong to EMRSA-15 clonal complex (CC) 22 and sequence type (ST) 22, which is a community, acquired MRSA type IV become frequent in many hospitals that represents 77% in the UK and Ireland (14). In north of Portugal this clone represents 68% of diabetic foot ulcers (15). Whereas other study in Tangier hospital in Morocco showed a prevalence of 52.94% of nasal MRSA colonization belonging to sequence type (ST) ST22 (16).

The second most frequent clone is the epidemic clone EMRSA-16 corresponding to clonal complex (CC) 30 (ST36) also known as USA200 widespread in UK hospitals and represents 14% (14). In other study this clone represents 65.4% of surgical and transplantation ward patients. These controversial observations highlight the need of further studies regarding the involvement of EMRSA-16 clones in healthcare settings. For CC1, CC5 and CC8 clones were estimated in a range of 1% to 3%, according to investigation established in England, Northern Ireland(14).The CC398 was mainly described as livestock associated which is a common colonizer of healthy animals especially pigs. This clone could be transmitted to human through animal exposure and via the food chain (17).

Types of MRSA

Most MRSA strains infections, was first associated to prior and prolonged exposure to hospital and or health care environments, antibiotic use, intensive care, individuals with HIV or cystic fibrosis, and contact with individuals with MRSA infection. They are highly detected among children and elderly patients hospitalized that were treated with antibiotics. These strains were classified as nosocomial or healthcare associated MRSA (HA-MRSA) (18, 19).

MRSA infections have been identified in healthy individuals, leading to the emergence of new strains known as community-associated MRSA (CA-MRSA). Consequently, MRSA is no longer only a nosocomial pathogen but recognized as a distinct clinical strain community-associated. Thus, two infections reservoirs were recognized in both health care and community settings (18, 19).

Although, such a distinction can be confusing, typing approaches have made it possible to categorize these two types of MRSA and have shown clear differences in phenotypic and genetic characteristics (19).
Then, MRSA colonization has been reported in a wide range of animal species, revealing new reservoirs of MRSA strains, referred as Livestock-associated MRSA (LA-MRSA) (20).

Healthcare-Associated MRSA (HA-MRSA)

Since 1980s MRSA infections incidence rates has increased gradually in many parts of the world, first appeared in the United States of America and in 1990s in the UK (18). MRSA become then endemic in hospital settings which constitutes a global problem in university hospitals, and in smaller healthcare facilities creating a great struggle for the healthcare system in order to find an efficient control strategy. MRSA strains exhibit a high level of resistance to several antimicrobial agents and can cause a range of infections such as pneumonia, bacteremia, and invasive infections.
HA-MRSA strains were typically resistant to clindamycin and other non-β-lactams antibiotics. Molecular evidence suggests that most of HA-MRSA strains carry SCCmec elements belonging to type I, II or III (18).
Among the HA-MRSA strains commonly identified in US Hospital SCCmec type II elements are the most frequent, while SCCmec type III elements are commonly identified in other countries (18). For example, in Iran, a study showed a high frequency of SCCmec type III (66.4%) among tested MRSA isolates (21).

Community-Associated MRSA (CA-MRSA)

The epidemiology of MRSA infections has changed with the emergence of a new strains of community MRSA infection in healthy subjects with no prior exposure to healthcare settings.
They become a serious problem in USA with the appearance of very infectious and virulent clone USA 300, which corresponds to sequence type 8 (ST8), causing several severe clinical syndromes such as necrotizing pneumonia and sepsis. Also, the USA 300 strain has been reported in Europe corresponding to CC80 (ST80) (18).
The CA-MRSA have rapid dissemination among general population and have different epidemiological, genotypic and clinical characteristics from HA-MRSA (18). Indeed, CA-MRSA have different staphylococcal cassette chromosome *mec* (SCC*mec*) elements, affect different populations outside health care settings, and cause different clinical symptoms than those of HA-MRSA (22).
Molecular typing methods have demonstrated that most of CA-MRSA strains carry smaller SCCmec types IV and V that are susceptible to narrow-spectrum of non-β-lactam antibiotics such as clindamycin, trimethoprim-sulfamethoxazole, and tetracyclines. They typically carry genes coding for the Panton–Valentine leukocidin (PVL) cytotoxin which is absent from HA-MRSA strains (18, 19). The PVL encoded by two genes *LukS-PV* and *LukF-PV* on mobile genetic elements (MGEs) are responsible of CA-MRSA strains virulence (18, 19). Thus, CA-MRSA strains are considered to be more pathogenic than HA-MRSA strains.
Some studies have shown evidence of CA-MRSA infections spread into hospitals, particularly in the United States but also in other countries (18, 19). An American study established during the period between 1999 and 2004 showed an increase in HA-MRSA strains of the SCCmec type IV (which is typical for CA-MRSA strains) from 20% to more than 50% (23).

Livestock-Associated MRSA (LA -MRSA)

MRSA has been found in different animal species and may represent a zoonotic transmission risk to humans (24). MRSA infection was first detected in 1972 in animals, in milk from Belgian cows that causes mastitis, and then was reported in different food and animals such as in pigs (24).
The widespread use of antimicrobials in human therapy, animals and agricultural settings has played a major role in the emergence of new MRSA strains. Therefore, MRSA infections are not only a human and community concern but also extend to veterinary medicine that threaten

companion, food animals and even wildlife animals (9).

Numerous studies have indicated a wide spread of LA-MRSA infections among pigs and pig farmers considered as an important source of LA-MRSA. MRSA colonization occurs in about 40% of pigs, 20% of cattle and 20 to 90% of turkey farms in Germany (9, 25-27). In addition, other studies have indicated a high risk of transmission of LA-MRSA infections in humans which are in contact with livestock. The overall prevalence of infected pig workers varies from one study to another but showed a significant increase in zoonotic transmission, around 29.7% in Spanish farmers, 32% in the Netherlands, up to 60% in Australia and 64.7% in Southern Italy (17, 25,26, 27).

The most relevant livestock-associated lineage is the ST398 (CC398) has been associated with zoonotic transmission in France since 2005. The ST398 lineage is widely spread among animals, and subsequently among healthy farmers where they are at a higher risk of having nasal *S. aureus* colonization (20).

Over the following years, there was an epidemiological spread of the CC398 strain found in several countries such as in Asia, Australia and America (13). This ST398 lineage is not only pig limited reservoir but has also been reported in domestic and even in wildlife animals. A recent epidemiological study on the prevalence of LA-MRSA in 1242 human MRSA isolates on North-West of England showed a low prevalence of (CC398) and mecC clones in humans (13).

MRSA Transmission

Several studies showed the presence of identical isolates in human and animals (Livestock and companion animals) suggesting a possible transmission pathway of bacteria or bacterial genes (2, 8, 22, 26).

S. saureus pathogen transmission depend on the expression of secreted and cell surface-associated virulence factors. These factors may act on three levels: promoting adhesion to host extracellular matrix components, altering host cells and corrupting the immune system (8).

HA-MRSA transmission involves several pathways, including surface contact, aerosols, hand hygiene, contact with healthcare personnel. Hygiene compliance is the main effective way to prevent transmission of MRSA infection inside healthcare settings (Fig.1).

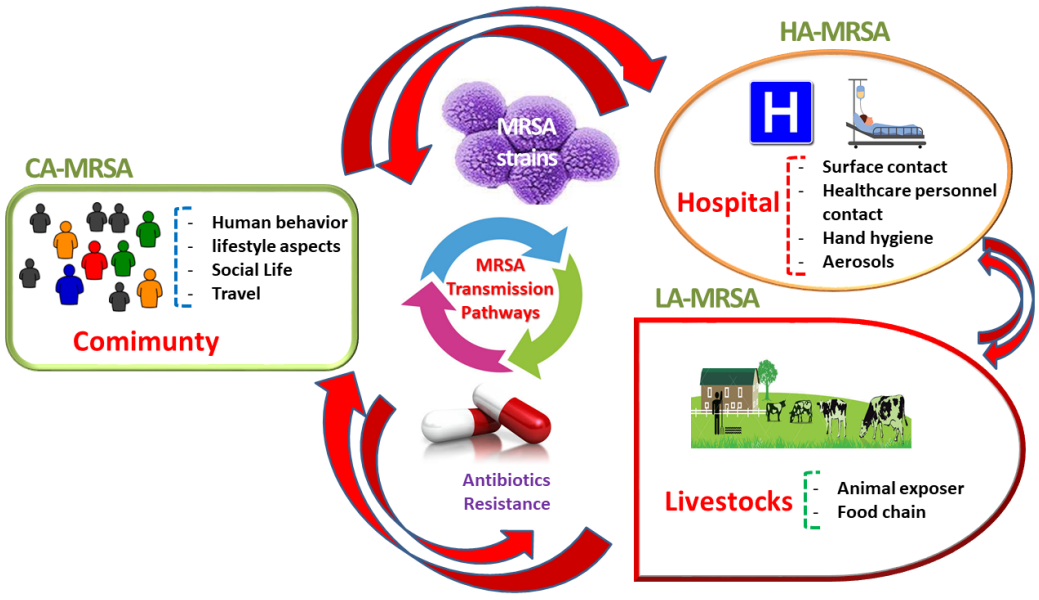

Fig. 1: Possible transmission pathways of MRSA between different reservoirs (Original)

Several studies focused on HA-MRSA while the main risk can be derived from CA-MRSA since it is considered as an important reservoir and may outbreak trough hospitals and generating more pathogenic strains (18, 19, 22). The CA-MRSA transmission follow a complex models trough community involving lifestyle aspects like travel, human behavior, and social life.

A careful monitoring of CA-MRSA is required since it is a crucial reservoir of resistant strains.

An estimated prevalence of 60% of known infectious diseases and up to 75% of new or emerging infectious diseases are of zoonotic origin (28).

The zoonotic transmission has several possible pathways, it can occur either by direct contact, between colonized or infected animals and humans via blood or other body substances during diagnostic or treatment, or indirectly through contaminated equipment or through vectors (such as fleas or insect), or through food or water contamination (9). Pathogen reservoirs may extend also in the environment outside their animal host leading to other transmission pathways such us air, dust, and manure (2, 9).

The most common strain of MRSA known to be transmitted by direct contact between animals and humans is CC398. Therefore, the nasal colonization of LA-MRSA CC398 represents up to 86% of humans occupationally exposed to pigs in German farming settings. The extension and persistence of this colonization seems to be tightly correlated to the frequency, intensity and duration of animal contacts (29).

Alternative MRSA treatments

Today, MRSA infections are still causing a growing global concern due to the rapid adaptive multidrug resistance to treatments with conventional antibiotics (5-9). Therefore, it is necessary to find new and alternative methods to overcome resistance and to obtain effective treatments against these MRSA infections for better clinical outcomes. The synergic use of two antibiotics or antibiotics with adjuvants appears to be a promising therapeutic approach.

In this context, results of some reports on MRSA agents suggested a better efficacy or bactericidal effects of rifampicin plus sulfamethoxazole-trimethoprim with vancomycin compared to the use of vancomycin alone (30, 31).

Different studies highlighting a superior activity of phytochemicals against different bacterial infections with negligible side effects compared to chemicals drugs (30, 31). Phytochemicals consist of different groups including polyphenols, flavonoids, terpenoids, and glycosides.

The use of combination therapy of antibiotics with phytochemicals is more effective than using antibiotics alone. The tested phytochemicals (tannic acid and quercetin) in combination with antibiotics showed a bactericidal activity against MRSA infections with maximum killing rate after 24 hours of incubation (32).

Bioactive plant products such as *Bauhinia kockiana* flower have showed antibacterial activity since plants develop a defense system through the synthesis of secondary metabolites which play an important role in their resistance to pathogens and microorganisms (33). In fact, the gallic acid and methyl gallate phytochemicals derived from *B.kockiana* flower by ethyl acetate extraction exhibit a strong antibacterial activity towards MRSA strains. The minimum inhibitory concentration (MIC) ranges of these extracts were estimated to 250–500 µg/mL. The Scanning electron microscopy investigation revealed that this extract may lead to bacterial cell membrane plasmolysis (33).

An important antibacterial activity has been observed using essential oils derived from Oregano vulgare (*O. Oregano*) against several MRSA nosocomial strains. Effective results against most the clinical isolates in particularly nasal swab isolates were noticed with MIC of 0.5 µg/ml (34).

Another study showed an important activity of thyme oil and trans-cinnamaldehyde against MRSA by reducing respectively the biofilms mass 59.7–85% and 52.9–82.4% and biofilm metabolic activity 79.3–86% and 85.9–88.7% after 48 hours treatment at 0.1% concentration (35).

The rhodomyrtone a plant-derived principal compound isolated from Rhodomyrtustomentosa

(Myrtaceae) leaf extract could be an alternative option for existing antibiotics for the treatment of MRSA infections (36). A significant antibacterial activity of Methicillin and other Gram-positive resistant bacteria after 4 hours treatment with rhodomyrtone extract have a MIC (0.5–1 μg/mL). At higher concentration of rhodomyrtone (4× MIC), the macromolecular synthesis assay revealed an inhibition of macromolecule synthesis after 30 min (36).

The phytochemicals may act on *S. aureus* via several mechanisms including hampering the permeability of the membrane, by inhibiting efflux pumps multidrug resistance, and inhibiting the β-lactams (37).

A promising therapeutic antimicrobial agents tested against MRSA consist in combining phytochemicals with nanoparticles including gold, silver, copper (38).

The synergic use of nanoparticles linked with phyto constituents represents a new trend of medical therapy due to their small size, large surface area, the ability to target specifically varieties of surface and to penetrate physiological barriers. The nanoparticles have a prominent future in modern medicine, due to their ability to enhance the half-life of bioactive molecules for efficient treatments (38).

Biogenic phytochemicals made of plants extracts (cassinopin and isoquercetin) capped with copper nanoparticles have an antibacterial and anti-biofilm effects. This biogenic phytochemical allowed a reduction of more than 50% of biofilm formation by MRSA (38).

A phytochemical nanoparticle by self-assembly of berberine and 3, 4, 5-methoxycinnamic acid derived from herbal Chinese medicine exhibit a good MRSA antibacterial activity compared to conventional antibiotics amoxicillin, norfloxacin. The obtained nanoparticles display bacterial inhibition rate of 94.62% at 0.1 μmol/mL concentration after adhering to bacterial surface (39). Another important antibacterial effect was obtained using self-assembly nanoparticles derived from berberine and cinnamic acid by both surface adhering and infiltration on bacteria cell leading to converging attack against MRSA, the inhibition was estimated to 95.03% with important ability of biofilm removal (40).

A combined effect anticancer and anti MRSA have been reported using silver nanoparticles with Rheum ribes extract. The antimicrobial effect was observed on gram-positive *S. aureus, MRSA, Bacillus subtilis* and gram-negative *Escherichia coli* bacteria. At a concentration of 200 μg/mL the lethal effect against MRSA was estimated to 48.96% (41).

Conclusion

The MRSA resistance remains a major healthcare issue, despite the wide progress, approaches to identify and characterize these bacteria and the new affordable treatment through pharmacological progress and bioactive phytochemicals. The genetic adaptation and expansion of MRSA trough community and livestock make extra reservoir for these strains that is challenging to face.

The wide spread and overuse of antibiotics has led to the immergence of more resistant bacteria. Despite progress and trials to fully understand the resistance mechanisms, MRSA remains the leading cause of nosocomial infections that cause death in vulnerable patients in absence of early identification of the infection or the late implementation or adequate treatment. Also, the spread of these infections trough community and livestock have socio-economic and health impact. Therefore, efficient preventive strategies are highly required add to the identification and development of novel drugs or active biomolecules through phytochemicals are time challenging to face new resistant strains.

Future research should focus on new alternative treatments, as well as on the application of good practice guidelines and biosecurity measures in each setting (hospitals, farms, abattoirs and food processing units) will certainly help in effective control to reduce colonization or the spread of MRSA infections in humans and animals.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declare that there is no conflict of interests.

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