



Review Article

A review on Methicillin resistance in *Staphylococcus aureus* in dairy cows and its consequences

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ABSTRACT

Staphylococcus aureus is a notorious gram-positive bacterium that presents life-threatening consequences by affecting both humans and animals. Methicillin is a semisynthetic antibiotic that is used as an antibacterial, especially for *S. aureus* and infections caused by *S. aureus*. Methicillin-resistant is a significant risk to world health, *S. aureus* (MRSA) has unique categories that encompass strains connected to hospitals, communities, and animals. In densely populated developing nations, the challenges related to public hygiene practices can be particularly pronounced. Developing nations, unfortunately, documented cases of both MRSA within healthcare settings and a concerning rise in the incidence of MRSA, posing a significant and evolving threat to the wider community. MRSA is a significant contributor to dairy cow mastitis, and this includes instances of MRSA. What complicates matters further is the reported transmission of MRSA between those handling milk and the cows themselves. This phenomenon is not unique to Asian continents but has been monitored on a worldwide scale, with similar reports emerging from within the country. These findings underscore the potential for MRSA being imparted within animals and humans, particularly those engaged in the dairy industry.

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Introduction

Staphylococcus aureus stands out as a prominent opportunistic pathogen, significantly impacting both human and veterinary medicine. This versatile bacterium poses a substantial threat to animal health and can potentially facilitate transmission between animals and humans, leading to a considerable burden on animal welfare and substantial economic losses in the livestock

industry. The prevalence of MRSA in livestock is higher in Asia as compared to other continents (Prakoso et al. 2023). Consequently, there has been a growing focus on addressing this pathogen in both livestock and companion animals (Algammal et al. 2020). In the realm of food safety, *S. aureus* ranks as the third most significant food-borne pathogen globally, thus challenging both public and animal health. Furthermore, certain strains of

humans can become harmed from eating enterotoxigenic *S. aureus*; it is frequently spread by milk products (Roshan et al. 2022).

It's important to recognize that a significant zoonotic pathogen, *S. aureus*, causes a variety of infectious illnesses that can harm both people and animals (Naseer et al. 2018). This bacterium not only inflicts substantial economic losses in livestock production but also represents a looming threat to public health (Zhou et al. 2017). *S. aureus*, commonly referred to as *S. aureus*, holds the distinction of being a prevalent human infection that gives rise to a range of ailments in medical settings such as hospitals. Consequently, it has become a subject of increasing concern within the global public health community (Lamer et al. 2011). One major cause of disease and death worldwide is thought to be methicillin-resistant *S. aureus* (MRSA) (Nadi et al. 2023).

S. aureus is a widely recognized pathogenic bacterium with a notable history of causing epidemics within healthcare environments among human populations (Wang et al. 2023). Additionally, it is an essential issue in the major cause of the inflammation of udder in cattle, highlighting its influence on both animal and human health (Moon et al. 2007). This pathogenicity is attributed to an order of virulent factors it possesses. These factors encompass biofilm formation, often referred to as the "slime factor," along with a variety of proteases, lipases, and elastases. Panton-valentine leucocidin (PVL) is also armed with an array of enzymes. These virulence elements collectively enable *S. aureus* to inflict damage to host tissues and facilitate its ability to spread to other anatomical sites (Gordon et al. 2008).

In clinical settings, semi-synthetic penicillin medications, including methicillin, are frequently utilized in the treatment of *S. aureus* infections (Otarigho and Falade 2023). However, the geography of *S. aureus* infections took a significant turn in the 1960s with the emergence of methicillin-resistant *S. aureus* (MRSA) (Jevons 1961). The main factor causing MRSA development is the *mecA* gene, which is often located on the movable genetic element called the staphylococcal cassette chromosome *mec* (SCC*mec*). Several remarkable SCC*mec* kinds have been reported, including SCC*mec* types I, II, III, IV, V, and VI reflecting the genetic diversity underlying MRSA strains (Deurenberg et al. 2007). Furthermore, these SCC components have additionally detected in other *Staphylococcus* species, expanding the scope of antibiotic resistance concerns to include *S. sciuri* (Juuti et al. 2005), *S. hominis* (Katayama et al. 2003), *S. epidermidis*, *S. haemolyticus*, and even within *S. aureus* itself (Hanssen et al. 2007).

Encoded by the *mecA* gene, Penicillin-binding protein 2a (PBP 2') is a modified penicillin-binding protein. Inside the bacterial cell wall, this protein is

positioned strategically and has a lower affinity for beta-lactam antibiotics. Consequently, the efficacy of conventional therapy is compromised, rendering MRSA strains highly resistant to these treatment options. It's crucial to recognize that apart from genetic factors, the emergence and persistence of MRSA are influenced by several other variables. These include inappropriate antibiotic usage, suboptimal dosing, and improper administration practices, all of which contribute significantly to the acquisition and proliferation of antibiotic resistance. MRSA has evolved into a formidable pathogen, not confined to healthcare-associated environments but also prevalent in community-associated settings (Klein 2007). In a disconcerting development, MRSA has also been evaluated from milk, indicating its involvement in livestock-associated infections (Devriese et al. 1975). This multifaceted distribution underscores the pressing need for comprehensive strategies to combat MRSA infections, considering its impact on both human health and agricultural practices.

Livestock associated with MRSA

Farm animals are now infected with (MRSA), including bovine and swine, also in pets, people who have intimate interaction with these animals, and companion vertebrates (Cuny et al. 2010). There have been reports of human infections connected to livestock-associated MRSA worldwide (Van Cleef et al. 2011). If antibiotic-resistant strains get into the community and healthcare facilities, they might represent a serious threat to the public's health (Fitzgerald 2012). The acknowledgment of bacterial transmission between people and cattle has grown recently, especially among farmers and farmworkers in Europe and industrialized countries (Chambers et al. 2009).

Beginning in the early 1970s, when MRSA colonization and diseases in dairy cattle were initially reported (Paterson et al. 2014). Notably, *S. aureus* linked to bovine mastitis has been shown to contain a type XI SCC*mec* with a highly divergent *mecA* gene, presently referred to as *mecC*. Various nations' mastitis MRSA strains have been shown to have various molecular characteristics. For instance, even though it is not the sole marker linked to mastitis, ST398 MRSA with type IV or V SCC*mec* has been implicated with clinical or subclinical bovine mastitis in certain European nations (Holmes et al. 2011). The *S. aureus* species-specific *nuc* gene hydrolyzes DNA and RNA in host cells, resulting in tissue damage and promoting the spread of *Staphylococci* (Abd-Elfatah et al. 2023). These findings indicate the presence of various MRSA clones associated with mastitis in cows worldwide.

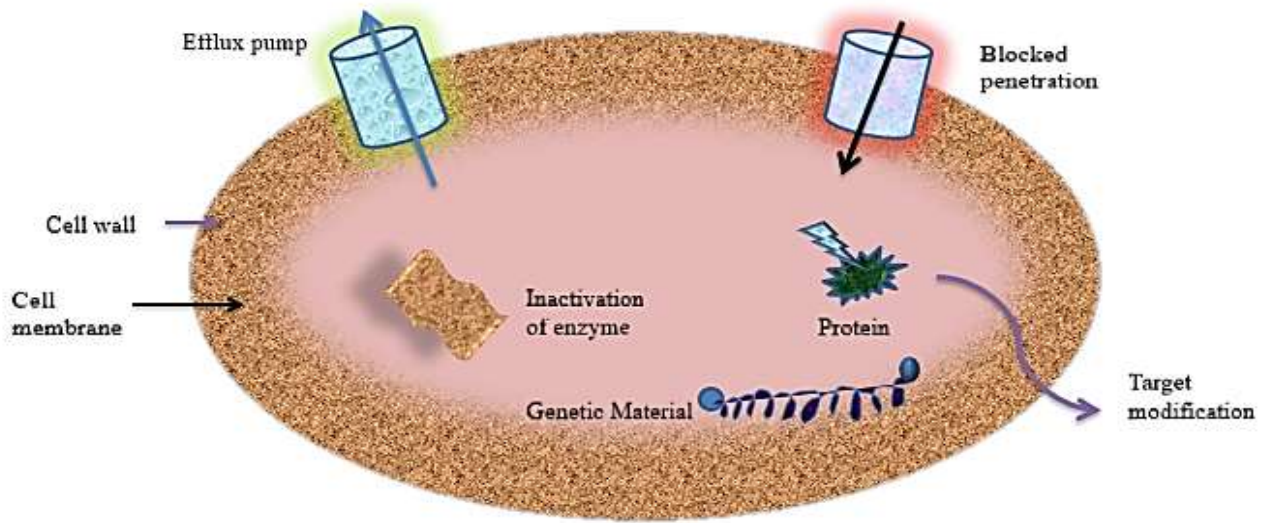


Fig. 1: Resistance shown by the *S. aureus* against Methicillin.

Table 1: Records of the transmission of mastitis-causing pathogenic strains to humans because of proximity, or food products.

Sr. No	Routes of Transmission	Pathogen/Strain	Location	References
1.	Milk	LA-MRSA	United Kingdom	(Paterson et al. 2014)
		LA-MRSA	Japan	(Sato et al. 2017)
		mecC MRSA	Great Britain	(Paterson et al. 2014)
		MRSA ST398	Belgium	(Schnitt 2022)
2.	Farm work / Direct contact	MRSA	Hungary	(Kos et al. 2023)
		MRSA	Denmark, United Kingdom	(García-Álvarez et al. 2011)
3.	Beef	MRSA	Korea	(Lee 2003)
		MRSA ST9	England	(Gelbíčová et al. 2022)
		MRSA	Japan	(Sato et al. 2017)
4.	Unknown transmission	MRSA ST398	Netherland	(Foley et al. 2022)

LA (Livestock associated), ST (Sequence types), MRSA (methicillin resistance *S. aureus*)

Methicillin resistance as measured by MRSA has been found to occur at a rate of 47.6% in *S. aureus* isolates from Chinese dairy farms (García-Álvarez et al. 2011). Given India's status as the largest milk

producer globally, dairy farming is a significant agricultural activity. The greater percentage 19.23% found in our investigation points to inappropriate use of antibiotics and insufficient intramammary

administration in cows with mastitis. Farm animals may operate as a reservoir for MRSA ST398 as human infection can occur via intimate contact with animals, which is the main risk factor (Garcia-Alvarez et al. 2011).

Many antibiotic resistance genes have been identified as originating in environmental microbes and subsequently transferring to other bacteria via mobile genetic elements, such as phages (Wright et al. 2010). These isolates were of ST9 spa type t899, lacking the PVL gene and carrying a type III SCCmec element. Another study from China identified MRSA strains in pet animals and veterinary staff. These isolates were identified from one another using the *mecA*-specific PCR assay, MIC testing, and the API Staph-Ident System (Zhang et al. 2005). Milk samples from cases of bovine mastitis contained MRSA in a variety of forms, including ST97 with SCCmec type IV, ST965 with SCCmec type IV, ST6 with SCCmec type IV, and ST9 with untypable SCCmec (Wang et al. 2012). There was no host preference among the animal species in Rio de Janeiro State, Brazil, where staphylococcal isolates were recovered from milk samples of cows, sheep, goats, and buffalo suffering from subclinical mastitis (Aires-de-Sousa et al. 2007). There has been evidence of MRSA in milk from semi-extensive dairy cow farming in northeastern Brazil, which suggests further study is necessary to ascertain the extent of these bacteria throughout various milk production methods (Oliveira et al. 2016).

Transmission of MRSA from animal to human Being

Antoci et al. (2013) examined the prevalence and genetic composition of methicillin-resistant *S. aureus* (MRSA) in bulk tank milk samples and the animals used by dairy farmers in the region of Ragusa, South-Eastern Sicily. According to the study, MRSA was detected in 44% of bulk tank milk samples, 61% of nasal wipes from cows, and 36% of human nasal swabs. It's interesting to note that the number of livestock units, there was a significant correlation between the percentage of positive cows on the farm and the presence of positive bulk tank milk samples with the incidence of MRSA carriers among people. Spohr et al. (2011) reported MRSA-positive milk 5.1–16.7% of dairy cows' samples, while Virgin et al. (2009) in bulk tank milk samples, MRSA was not found. Methicillin-intolerant is one of the most prevalent types of *S. aureus* that cause infections in people in hospitals and the community.

It makes sense to believe that enhancing cleanliness on dairy farms might lower the likelihood of MRSA transmission, given that practices related to milking hygiene have been connected to somatic cell counts, which rise when mastitis is present. The spread of mastitis infections, particularly *S. aureus* among cows, is

frequently facilitated by milkers' hands and milking clusters, which might be the source of this transmission. Prospective studies are required to further investigate the transmission of MRSA between animals and humans and to implement preventive measures. MRSA, also referred to as resistant staph or "superbug." MRSA is a high priority microorganism for future research and therapy, according to the World Health Organization (Khan et al. 2022).

Köck et al. (2009) discovered a university hospital's tertiary care patients were in contact with pigs were linked to an increased incidence of MRSA CC398 colonization. Apart from the standard risk factors associated with MRSA carriage, Harbarth et al. (2006) proposed evaluating to identify those who are more likely to get colonized by MRSA, hospital admission screening should consider contact with cattle as an additional risk factor. Such individuals could potentially introduce MRSA CC398 into healthcare settings and contribute to the importation of antimicrobial resistance. Prashanth et al. (2011) performed research to determine the genetic similarity between *S. aureus* isolates from Indian livestock and people.

Recently, samples of ambient water and wastewater treatment plants have been shown to contain methicillin-resistant bacteria. Given that people use numerous antibiotics, wind up in wastewater, there is a worry that antibiotics might exert selective pressure and cause resistance genes to arise and spread in vulnerable species (Goldstein et al. 2012). Furthermore, it appears that phages function as environmental reservoirs for resistance genes since bacteriophages DNA extracted from ambient water samples contains the *mecA* gene from MRSA and the *E. coli*, β -lactamase genes (*bla*TEM and *bla*CTX-M9). This suggests that drug resistance in the environment may be facilitated through mobile genetic elements such as plasmids, transposons, or bacteriophages that transmit genes horizontally (Shetty et al. 2023).

Bovine Mastitis: Zoonotic Pathogens and Host-Immune Evasion

One prominent instance of "zoonotic spillover" is mastitis, in which a zoonotic pathogen aids in the spread of infection from a host reservoir for the surrounding area (Rodriguez et al. 2020). The potential for zoonotic overflow is dependent on several aspects, like the mechanism of illness in the host reservoir cows with chronic subclinical mastitis (SCM), pathogen exposure (bedding, parturition, injury of teat canal), and human aspects that impact exposure to contaminations (open wounds, inappropriate dressing, or close contact with animals).

Table 2: Dairy farms were impacted by reports of antibiotic resistance in mastitic milk.

Organism Type	Gene Type	Country	References
<i>Staphylococcus hyicus</i>	mecA	Finland	(Taponen et al. 2022)
MRSA	SCCmec type IVg	Korea	(Schnitt 2022)
MRSA	mecA	Brazil	(Rabello et al. 2007)
CoNS	mecA	European countries	(Hendrikse et al. 2008)
CoNS	mecA	UK	(Sheet 2022)
MRSA	Type IVa SCCmec-mecA	Pakistan	(Jamil and Rasheed 2023)
CoNS	mecA	Europe	(Fessler et al. 2010)
ST239/spa-type t30 ST8	SCCmec type III	Turkey	(Gopal and Divya 2017)

CoNS: coagulase negative; MRSA: Methicillin-resistant *Staphylococcus aureus*; SCCmec: Staphylococcal cassette chromosomal mec; ST: sequence type.

Pathogen surveillance primarily focuses on human clinical samples, and minimal research investigates animal strains. This bias can lead to the underreporting of incidents that occur when people and cattle interact, or vice versa (Alexander et al. 2018). *Staphylococcus aureus* and *Streptococcus agalactiae* are the significant infectious bacteria that are known to cause spillover and chronic intramammary infections; however, several studies have also included *Corynebacterium*, and *Streptococcus uberis* (Chehabi et al. 2019). Conversely, environmental infections such as *S. dysgalactiae*, and *Escherichia coli* seldom create outbreaks, unless they induce severe clinical signs in animals that are afflicted (Dufour et al. 2019). Geographic variance, variations in antibiotic

concentration, and the usage of different antibiotics could all contribute to the variations in antibiotic resistance among the various countries (Azam et al. 2023).

Mastitis milk can be a source of foodborne toxins and bacterial contamination, which puts human health at serious risk. Coliform bacteria, such as *E. coli*, are the cause of clinical mastitis (CM) is less relevant in zoonotic terms because abnormalities in milk are visible, leading to its prompt rejection (Nagasawa et al. 2019). In contrast, (SCM), where cows appear healthy, carries a higher potential for zoonotic transmission. There have been cases when bulk milk supplies have been contaminated with milk that doesn't show any symptoms (Rodriguez et al. 2023), resulting in the potential for human infection when ingested uncooked or with insufficient pasteurization.

Spillover transmission has been documented in staphylococcal infections, presenting a widespread and challenging problem to control because of several pathogenic elements that are accountable for adhesion, colonization, invasion, and infection within the host. Since its first appearance in humans in 1961, the advent of special several (MRSA) strains have been a major factor in zoonotic transmission (Van Cleef et al. 2011).

Hospital-associated, community-associated, and livestock-associated are the three types of MRSA that have been discovered. Livestock associated MRSA has been identified a significant percentage of cattle on numerous farms across Europe, ranging from 32 to 37% on 40 to 45% of farms (Goerge et al. 2017). Several papers have covered other Livestock associated-MRSA sequence types (STs), including ST398, CC130, and others (Chih-Jung Chen et al. 2018; Tegegne et al. 2019), which were initially thought to be specific to cattle but have also been found in human clinical isolates.

Through direct contact or environmental conditions, humans and different agricultural animals are exposed to a constant flow of MRSA lineages both within and between farm habitats, as indicated by molecular studies comparing these sequence types (Locatelli et al. 2017). This emphasizes how agricultural animals can serve as carriers of infections to people and other animals. However, it is not always possible to determine with certainty transmission across species is taking place (Nazarov et al. 2021).

Soft tissue, cutaneous, or nasal infections are the most typical presentations in medicine of human MRSA infections. Furthermore, when people ingest contaminated milk, *S. aureus* strains create heat-resistant enterotoxins that can cause symptoms including nausea, vomiting, and cramping in the abdomen (Cuny et al. 2015).

Another significant zoonotic pathogen, frequently associated with subclinical mastitis (SCM) in Europe, is *Streptococcus agalactiae*. Multiple sequence types have been linked to asymptomatic

carriage in both cattle and humans, highlighting the diagnostic challenges (Chih-Jung Chen. 2018; Kayansamruaj et al. 2019; Lyhs et al. 2019). *S. agalactiae* is mostly linked to bacteremia, urinary tract infections, skin, and soft tissue infections, and, on rare occasions, more serious illnesses as meningitis, arthritis, toxic shock syndrome, necrotizing fasciitis, or endocarditis in adult humans (Lyhs et al. 2019). Numerous investigations have looked at the evolutionary relationships and genetic relatedness of various isolates of *S. agalactiae* from people and cattle. Understanding the role of these zoonotic infections in the worldwide health system is critical to understanding the spread of inflammation. Of particular significance is the potential for SCM, as cows appear healthy, yet food products like milk and meat can introduce food poisons and microorganisms entering the food chain.

Monitoring strategies to avoid the distribution of diseases resistant to antibiotics

It is essential to adhere to public health, hygiene, and sanitation practices. Culturally sensitive awareness campaigns should be developed and targeted towards the public, stressing the need to save antibiotics and only use them when absolutely required. Educational efforts should focus on fundamental hygiene practices like handwashing to prevent the spread of infections. Improving sanitation systems is also essential to get rid of germs that are resistant to antibiotics in wastewater (Economou et al. 2015). Susceptibility of *S. aureus* was checked by the method of disk diffusion and antibiotic disks used are amoxicillin, ceftriaxone, and tetracycline (Mwafy et al. 2023).

To reduce antibiotic resistance within the domain of veterinary medicine, it is important to consider certain of the World Health Organization's (WHO) recommendations that are followed for human treatment. When an infection's etiology is identified using trustworthy microbiological techniques, antimicrobial medication should be customized to specifically target that organism. Recommendations for diagnostic tests, including microbiological, pathological, hematological, and biochemical data and values, should be part of standard treatment guidelines. Generally, recommendations lean towards a class of antibiotics rather than a single medication unless there is enough evidence to support a specific medication choice.

Economic Challenges by *S. aureus* mastitis

One of the foremost economic challenges associated with *S. aureus* mastitis is the significant reduction in milk production among affected cows. This not only leads to decreased milk volumes but also adversely impacts milk quality, potentially resulting in lower prices for dairy products (Richardet et al. 2023). As a result, the farm's revenue is directly

affected by these compromised milk yields. Additionally, the financial burden extends to increased veterinary expenses that farmers and livestock owners must bear. This includes costs related to veterinary services, diagnostic tests, and treatments necessary for managing *S. aureus* mastitis cases. Expenses associated with antibiotics, intramammary treatments, and veterinarian consultations further exacerbate the economic strain (Brock and Schewe 2023). In a previous study it was assumed that farmers take the infection from animals when they present with them by acquiring *S. aureus* (Liu et al. 2022).

In addition to quantity and quality issues, *S. aureus* mastitis contributes to decreased milk quality by elevating somatic cell counts (SCC) in the milk, indicating increased levels of white blood cells due to inflammation. Elevated SCC levels can lead to penalties or reductions in the prices paid for milk by dairy processors, adding another layer of financial stress (Penati 2023). Severe cases of *S. aureus* mastitis often require the culling of infected cows from the herd to prevent the spread of infection to other animals. The loss of a productive cow due to culling represents a significant economic setback for the farm, disrupting overall production capacity and potential revenue (Santos et al. 2019). Additionally, managing *S. aureus* mastitis demands extra labor inputs, involving additional time spent on milking, administering treatments, and closely monitoring affected animals, all of which translate into increased labor costs (Cameron et al. 2016). Furthermore, the use of antibiotics to treat *S. aureus* mastitis poses a looming risk of contributing to antibiotic resistance. This development can have far-reaching consequences, including increased healthcare costs and potential restrictions on the use of specific antibiotics, adding yet another layer of economic complexity (Singer et al. 2012). Beyond the financial realm, *S. aureus* mastitis also raises concerns about animal welfare. In addition to its economic implications, the disease can cause pain and suffering in affected cows. Mitigating this suffering may necessitate additional investments, further straining the economic aspects of dairy farming (Santos et al. 2019). Lastly, farms grappling with a history of *S. aureus* mastitis are at risk of suffering a loss of reputation within the dairy industry. Potential buyers and consumers may exercise caution when considering products from such farms, impacting market access and overall profitability (Wilson 2017).

Conclusion

In conclusion, effective strategies for limiting the transmission of MRSA infections encompass a range of measures including the detection and decontamination of asymptomatic MRSA carriers, patient education, and general infection control procedures, in both community and clinical settings. The spread of MRSA among animal

populations may also be significantly decreased by putting strong biosecurity measures, good husbandry practices, and basic hygiene practices into farms, slaughterhouses, and food processing plants. Those who have regular contact with animals should be made fully aware of the possible hazards related to MRSA transmission through animals or their surroundings. To regularly track the prevalence and evaluate the hazards presented by newly developing MRSA strains, urgent global activities are required. New research on community-associated MRSA indicates that some clones may be locally transmissible in hospital and community settings, especially following importation from the Asian subcontinent. Thus, it makes sense to consider screening for Gram-positive and resistant Enterobacteriaceae in patients who have been abroad or had medical contact, and to implement the appropriate infection control protocols afterward. A major obstacle facing healthcare is the widespread and careless use of antibiotics, as well as the developing threat of antibiotic resistance. Along with the cautious and responsible application of currently available anti-infective medicines in Pakistan and throughout the world, immediate action is necessary to address this issue. Additionally, since *S. aureus* transmission is a dynamic process involving animals, human beings, and maybe the environment for farming production, it is imperative to continue closely monitoring the bacteria's level of resistance in dairy environments. To pinpoint the crucial locations that encourage contamination and spread in the agricultural setting, more investigation is necessary.

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Availability of data and material

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Consent to participate

All the authors gave their consent for equal participation.

Consent for publication

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Competing Interest

The authors declare that they have no relevant financial or non-financial interests to disclose.

Author Contribution

MA, GM, WQ, TA, and MSA wrote the article MS, AMAK, and SA designed the picture and tables, All the authors managed references and correction.

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