Kufa journal for Veterinary Medical Sciences Vol.(11) No.(2)
Kufa journal for Veterinary Medical Sciences
Kj.vs@uokufa .edu.iq
www.uokufa .edu.iq\journals\index.php\kjvs

An overview: the virulence capacity, resistance mechanism, integron association, biofilm formation ability, and methicillin- resistance traits among staphylococcus aureus

Anas E. Almousawi, *Abdullah O. Alhatami **Furkan Alaraji
*Department of Public Health , Faculty of Veterinary Medicine, University of Kufa, Iraq
**Department of Pathology and Poultry Diseases , Faculty of Veterinary Medicine, University of Kufa, Iraq

Received date:2 Sept.2020 Accepted:(471) 1 Oct.2020 page: (23-41) Published:30Dec.2020

DOI: https://doi.org/10.36326/kjvs/2020/v11i23288

Abstract

The ability of *Staphylococcus aureus* (*S. aureus*) to acquire variety and many virulence genes that leads to its the ability to cause different diseases in many hosts species, these bacteria have many mechanisms for antibiotic resistance and their ability to produce biofilm and gain various genes through integrons, that will lead to difficult treatment. Consequently, the acquisition of the mecA gene made it more virulent and resistant to antibiotics, and this indicates that the spread of these bacteria in human and animal communities besides health institutions and their frequent transmission between these communities may have a problem of dealing with it.

Keywords: S. aureus, mecA gene.

نظرة عامة: قدرة الضراوة ، آلية المقاومة ، ارتباط إنتيجرون ، قدرة تكوين الأغشية الحيوية ، وخصائص مقاومة الميثيسيلين بين المكورات العنقودية الذهبية

انس اسماعيل الموسوي عبدالله عبيس الحاتمي فرقان الاعرجي

الخلاصة:

إن قدرة Staphylococcus aureus على اكتساب العديد من الجينات الضراوة أعطتها القدرة على إحداث الأمراض في العديد من الكائنات الحية ، إلى جانب أن هذه البكتيريا لديها العديد من الآليات لمقاومة المضادات الحيوية وقدرتها على إنتاج الأغشية الحيوية واكتساب جينات مختلفة من خلال integrons ، مما يجعل العلاج صعبًا للغاية. بالإضافة إلى ذلك ، فإن اكتساب الجين mecA يجعلها أكثر ضراوة ومقاومة للمضادات الحيوية ، وهذا يشير إلى أن انتشار هذه البكتيريا في المجتمعات البشرية والحيوانية إلى جانب المؤسسات الصحية وانتقالها المتكرر بين هذه المجتمعات قد يشكل خطرًا يصعب التعامل معه.

الكلمات المفتاحية: S. aureus، mecA gene.

introduction

The first observation and culturing of *Staphylococci* were done by Koch (1843-1910) and Pasteur (1822-1895), a few years later a Scottish surgeon named Sir Ogston (1844-1929) made the first detailed studies on this bacteria, He noticed under the microscope a cluster of round cells and described their causative role in the formation of an abscess, this genus of pus-forming bacteria named by Sir Ogston based on their appearance under the microscope (Ogston, 1882; Ogston, 1984; Licitra, 2013), the first isolation and growing of *S. aureus* species was by Rosenbach (1842-1923)

and according to the yellowish color of its colony named it as aurum that means gold (Rosenbach, 1884; Licitra, 2013). a few years later, Chapman (1930) introduced the tube coagulase test for differentiation of *S. aureus* from the less virulent staphylococci (5). In 1928, Alexander Fleming noticed that *S. aureus* couldn't grow with Penicillium mold in the same place (6), therefore, 10 years later large quantities of penicillin were purified to begin treatment trials (7,8). The genus *Staphylococcus* belongs taxonomically to the family Staphylococcaceae from the order bacillales member of the class of bacilli belonging to the phylum firmicutes, this classification was according to Phylogenetic relationships of the genus *Staphylococcus* constructed on sequence analysis of 16S rRNA gene (9). *Staphylococci* are gram-positive cocci organized in clusters that resemble a bunch of grapes (10). The *Staphylococcaceae* genus containing more than 80 species and subspecies, The coagulase-positive such as *S. aureus*, coagulase-negative such as *S. saprophyticus*, S. *epidermidis*, and the coagulase variable like *S. hyicus*, that causing infections for both humans and animals, indeed, the more interesting one is *S. aureus* that has spherical cell 0.5–1.5 μm in diameter (11,12). Macroscopically, it has a significant golden-yellow appearance that was found to be associated with increased virulence character (13). It grows will at 37C^{o} on routine laboratory media and its facultative anaerobe that grow in the presence of O_2 and CO_2 better with raised, opaque and round colony, many strains of *S. aureus* produce coagulase that differentiate it from other coagulase-negative *staphylococci* (14). *S. aureus* bacteria can grow between the range of temperature (15◦C-45◦C) and at high concentration of sodium chloride (NaCl) reach to 15 %. However, high temperatures above 45 degrees and a decrease under 10 Celsius degrees will affect on the growth of these bacteria naturally, In addition, these bacteria was resistant to high osmolarity and detergents such as alcohol and have the ability to ferment mannitol sugar, therefore, it grows on the mannitol salt agar that rich in NaCl and mannitol sugar. Moreover, *S. aureus* has the potential to grow on most culture media including Blood agar, Nutrient agar, Mannitol salt agar, Tryptic soy agar, Brain heart infusion agar, Luria Bertani agar, and MacConkey agar, Consequently, it has many characteristics on each culture medium, for example on the blood agar produced beta-hemolysis, golden colonies on nutrients and mannitol salt agar, and many other forms depending on the culture medium (15,16). To observe their morphology and unique colour, the typical colonies obtained on the media mentioned above are subjected to Gram staining. After that, the suspected isolates could be submitted to the catalase test for catalase enzyme determination and its activity, and confirm rabbit plasma clotting using coagulase tests to detect the bound and free coagulase proteins, In fact, a large number of automated systems and commercial kits based on mini biochemical tests are commonly used nowadays to identify this bacterial species in both diagnostic and research laboratories, including the API Staph system kit, the VITEK1 system, the VITEK2 system and many others, but the molecular methods remain the

golden standard in all fields of identification (17).

General pathogenesis and virulence factors

S. aureus is a natural colonizer of the humans and animals skin, it may cause several pyogenic and systemic infections, it's an intra- and extracellular microorganism, this characteristic is probably involved in bacterial tolerance, antibiotic safeguarding, and evasion of immune system defenses. Its genetic flexibility acts as an exciting ground for adaptation. *S. aureus* virulence factors were characterized by numerous pathogenic processes leading to host structures being adhered to, and targeted, accompanied by internalization, intracellular stability, and immune avoidance (18). This species could cause various cases of localized infections such as wound infection, carbuncle, and cellulitis, however, it can spread inside the bloodstream to different organs causing sepsis and many other serious systemic conditions like osteomyelitis, endocarditis, septic arthritis, and epidural abscess (19). *S. aureus* possess a huge number of virulence factors that facilitate the attachment to the host tissue, evading the host immunity, promote the tissue invasion, and induce toxicosis. Their characteristics, factors, and their functions are summarized in Table 1.

Prevalence of *S. aureus* in animals

S. aureus colonizes various biological tissues in different animals and humans in various conditions, as 30% of people are carriers of *S. aureus* bacteria, in cows, this percentage reaches 35% while may reach 90% in poultry, moreover, the animals can act as a reservoir for this pathogen and can transmit it to humans (20,21). Many reports suggested that the phenotypic properties of *S. aureus* species vary depending on the host of origin, six biotypes have been described that vary in their phenotypic characteristics including, β-hemolysis human, human, bovine, caprine, avian-abattoir, and non-specific host biotypes (22). the evolution of *S. aureus* with its human host over time gives it the ability to infect animals on multiple occasions, the ability to jump from one host to another will ultimately led to creating specific strain lineages that can spread and adapt within new animal hosts (23).

antibiotic resistance mechanisms

The main targets for antibiotics in the genus *staphylococci* were the cell envelope, ribosome, and nucleic acids. Resistance occurs either through mutation in chromosome genes or through horizontal transfer of resistance determinants encoded through mobile genetic elements such as transposons, plasmids, and the staphylococcal chromosome cassette (SCC). The trait of resistance can result from (i) preventing the drug from binding to its target by altering this target itself, (ii) activating chromosomally encoded multidrug resistance efflux pumps, (iii) reducing drug access to its target through multiple mutations which change the structure and composition of the cell wall and/or cell membrane, however, the mechanisms that responsible for horizontally acquired resistance result from : (i) alternation and inactivation of drugs by enzymatic effectiveness, (ii) enzymatic alternation of the drug binding site, (iii) efflux pump, (iv) acquisition of a novel drug-resistant target using bypass mechanisms, (v) protect the target by drug displacement. (24). The diversity of the mechanisms of

S. aureus resistance to antimicrobial drugs led to penicillin resistance within 10 years of its discovery. By the 1960s, more than 80% of *S. aureus* isolates had gained penicillin resistance, which led to the introduction of semi-synthetic penicillins. The first, methicillin in 1961, followed by other derivatives, such as oxacillin, cloxacillin, and dicloxacillin (25).

The emergence of methicillin resistance among *S. aureus*

Jevons reported a strain of *S. aureus* that was resistant to methicillin, soon after its discovery (26) an altered penicillin-binding protein 2α (PBP2α) with a low affinity for beta-lactam antibiotics has been encoded with the *mecA* gene which responsible for Methicillin resistance in staphylococci (27). several studies showed that MRSA is generated when Methicillin-sensitive Staphylococcus aureus (MSSA) acquire the *mecA* gene. Recently, a new homologue of *mecA* gene, called $mecA_{LGA251}$ in reference, to the *S. aureus* $_{LGA251}$ isolates from which it was described, it has also acted as methicillin resistance, it was renamed to *mecC* (Laurent *et al.*, 2012; arrison *et al.*, 2013; Petersen *et al.*, 2013; Paterson *et al.*, 2014). The *mecA* gene included a large mobile genetic elements designated as SCCmec (the staphylococcal cassette chromosome mec), MRSA strains are thought to have arisen through horizontally transferred SCCmec from coagulase-negative staphylococcus species (32). Since 1961, the spread of MRSA clones worldwide reaching an pandemic situation in most developed countries, it is not identified whether this is due to differentiation from just a single particular clone or the insertion of SCCmec into multiple clones (33). mainly, MRSA clones have emerged from healthcare-associated (HA) origin, but in the 1990s and the early 2000s in Australia, USA, and Europe, MRSA infections have been documented in people with no previous medical exposure, and these strains have been named as community-associated (CA) MRSA (34).

Table (1): Virulence factors of *S. aureus* and its characteristics (35)**.**

function	factors	characteristics
facilitate the attachment to the tissue of a host	Cell surface components	Involved in host immune evasion, this family of surface proteins which interact with host molecules such as fibrinogen and fibronectin, therefore, facilitating the attachment to host tissue. protein A, fibronectin-binding protein A and B, collagen-binding protein, and clumping factors A and B are the most common examples of this family of virulence factors.
Breaking/evading the host immunity	Polysaccharide microcapsule	surrounding the bacterial cell wall and has an anti-phagocytic activity.
	Protein A (SpA)	It has many biologic characteristics like anti-complement activity, chemotactic properties, as well as it is anti-phagocytic protein, limits the host immune response, induces hypersensitivity reactions and platelet damage, also it was amplified the natural killer activity of human lymphocytes, the (SpA) nonspecifically bind to the Fc domain of immunoglobulin and act as a superantigen.
	(PVL)	associated with CA-MRSA, causing leukocyte lysis and tissue necrosis that allow this pathogen to cause skin and soft-tissue infections (STIs), this toxin has a two-component slow (S) subunit and fast (F) subunit, which work together to induces pore formation in the cell membrane of leukocyte cells producing necrosis.
	α-toxin (α hemolysin)	This toxin has significant leucocytic properties and helping in the scavenging of iron in the cells, a pore former exotoxin causing leakage in the cell membrane of the host and death.
	Chemotaxis-inhibitory	An extracellular protein that inhibits neutrophils and monocytes chemotaxis

	proteins	activity.
Tissue invasion	Extracellular adherence protein	An extracellular protein binds to the host cell matrix, plasma proteins & endothelial cells. these proteins can stop the action of host defenses and promote adherence to tissues also promote the pathogenesis of staphylococcal abscesses, bacteremia, and endocarditis such as coagulase enzyme.
	Lipase, protease, Hyaluronidase, Staphylokinase, and phospholipase C	Extracellular enzymes which causing tissue destruction, and therefore helping in tissue penetration.
Induces toxinosis	Enterotoxins	It is a group of toxins that have emetic activity, the most common one is enterotoxin type A (SEA) that cause staphylococcal food poisoning worldwide, enterotoxin group are considered superantigen and encoded on mobile genetic elements (MGEs), including plasmids, pathogenicity islands, prophages, or by chromosomal genes located near the SCCmec element
	Toxic shock syndrome toxin -1	Its exotoxin has superantigen properties that cause dangerous consequences, characterized by high fever, rash, diarrhea, vomiting, and death, which was first recognized in women that use vaginal tampons. This superantigen causing shock by attracting large numbers of T lymphocyte to the site of infection leading to the release of a huge amount of cytokines.
	Exfoliative toxins A and B	Serine proteases which break down skin desmosomal proteins and cause staphylococcal scalded skin syndrome (SSSS), a disease especially affecting infant's

Staphylococcal Chromosomal Cassette mec (SCCmec)

A specific type of mobile genetic elements (MGEs) that codes for the resistance of methicillin's, it was detected in almost all MRSA strains. SCCmec elements integrate at the bacterial chromosome attachment site (attBscc) found nearby the origin of replication, at the 3' end of unknown function open reading frame X (orfX) (36–38). The attachment site also contains the integration site sequence (ISS) that consisting of a core of 15 base pair sequence, it was essential for cassette chromosome recombinase (ccr), ISS was found in direct repeat sequences at left and right of SCCmec junctions of the integrated SCCmec element, SCCmec elements share the similar arrangement of structure, that composed of two crucial parts, mec complex that composed of mecA operon and its regulators, and ccr gene complexes that encoding the site-specific recombinases, these two confer resistance for methicillin and the mobility of the SCCmec cassette, also these genes surrounded by three- highly variable-joining regions (J1 to J3) which may convey further resistance determinants to antimicrobial agents. The composition of almost all SCCmec elements shares the same general organization: orfX-J3-mec-J2-ccr-J1. (38–41).

Classification of SCCmec elements:

Typing or classification of SCCmec elements is based on the association between mec gene complex classes and ccr gene complex types, and each SCCmec type can be subtyping depending on variations in their J regions within the same cassette (39). In this context, *mec* genes complex classified according to regulatory genes (*mecR1* and *mecI)*, and insertion sequence IS*431* downstream of *mecA*, Certain variants of the *mec* gene complex contain insertion of either IS*1272* or IS*431* at the 3′ portion of mecR, so it's classified into six different classes (A, B, C1, C2, D, and E) (38,39,42), mentioned in Table 2. While the ccr gene complex that composed of the ccr gene(s) encoding for invertase-resolvase enzymes that can catalyze and/or insert of SCCmec into the chromosome of a Staphylococcus strain,

thus These recombinases are responsible for cassette mobilization, ccr complexes surrounding by open reading frames (ORFs), that have unknown functions. (38,43,44).

Table (2): mec gene complexes of *S. aureus* (39,45)

Mec complexes	Characterization	SCC mec types
Class A	IS431-mecA-mecR1-mecI	II, III, VIII
Class B	IS431-mecA-ΔmecR1-IS1272	I, IV, VI
Class C1	IS431-mecA-ΔmecR1-IS431(two of IS431 in one direction)	VII, X
Class C2	IS431-mecA-ΔmecR1-IS431(two of IS431 in opposite direction)	V, IX
Class D	IS431- mecA-ΔmecR1	-
Class E	blaZ-mecA$_{LGA251}$-mecR1$_{LGA251}$-mecI$_{LGA251}$	XI

It was classified into (A, B, and C) Based on allelic variations, one carrying two neighboring genes, ccrA, and ccrB, and the second carrying ccrC, both A and B genes have been typed into four and five allotypes respectively, designated as type 1 (ccrA1B1), type 2 (ccrA2B2), type 3 (ccrA3B3) up to type 8, these types have been identified based on their nucleotide similarity, on the other hand, ccrC has shown high nucleotide similarity and is assigned to only one allotype (ccrC1) (39,40,46).

Meanwhile, the joining regions (J), previously called "junkyard" regions, was classified as (J1, J2, and J3) according to their location within the SCCmec element, it is located in the same position in all SCCmec elements (J1-ccr complex-J2-mec complex-J3), anyway, these components are nonessential and may contain determinants for additional antimicrobial resistance, but the variation of these J regions are used for determining SCCmec subtypes (39,45). As discussed above, SCCmec elements classified into types and subtypes according to variants in their composition, therefore type I defined as (1B) because of type 1 ccr gene complex and class B mec gene complexes, the other types are defined as in table 3, and backbone structure organization of SCCmec elements in *S. aureus* will appear like in figure 1.

Table (3): SCCmec types (39,47)

ccr gene complex	mec gene complex	SCCmec type
1 (A1B1)	B	I
2 (A2B2)	A	II
3 (A3B3)	A	III
2 (A2B2)	B	IV
5 (C)	C2	V
4 (A4B4)	B	VI
5 (C)	C1	VII
4 (A4B4)	A	VIII
1 (A1B1)	C2	IX
7 (A1B6)	C1	X
8 (A1B3)	E	XI

SCCmec typing methods

Generally, the global spreading of MRSA is driven by the dissemination of several clones with a similar genetic heritage, several epidemiological studies revealed that multilocus sequence typing (MLST) and spa typing are needed for appropriate clone detection, as well as SCCmec typing (48). The SCCmec typing methods were developed along with the new descriptions of the SCCmec types and introducing the novel

techniques or approaches for their study. This can be differentiated by three different SCCmec typing techniques: methods based on multiplex PCR; methods based on the restriction enzymes digestion; and methods based on real-time PCR (47).

(i) restriction enzymes digestion methods: This method was of significant use for epidemiological studies before the structure of the SCCmec element was described, the microbial DNA is extracted and digested with specific enzyme and then the resultant fragment patterns are compared. There are actually some principles for the use of restrictive digestion enzymes in conjunction with PCR, such as multi-enzyme PCR-amplified fragment length polymorphism (ME-AFLP), or PCR amplification of the ccrB gene in combination with restriction fragment-length polymorphism (RFLP) in SCCmec typing method (49,50). It is, however, just a pattern-based typing which could be an interesting tool for pre-screening an extensive selection of strains, but is already not adequately sensitive to assign SCCmec type properly for epidemiological purposes as it recognizes no characteristic features for SCCmec elements already described, mec class description in combination with ccr class is important for proper SCCmec assignment. It appears the SCCmec typing scheme based on the digestion of restriction enzymes is therefore no longer superior. The most promising methods used today for PCR-based typing (47).

(ii) typing methods based on PCR: Different methods for investigating the mec gene complex have been developed**,** many methods are based on PCR mapping of cassette genetic elements like ccr complex, mec complex, and J region. There are other methods that include sequencing internal fragments of recombinant genes (51).

(iii) Real-time PCR based typing methods: This approach, based on the rapid molecular beacon real-time PCR assay, it's built on the concept of SCCmec types as a combination of the ccr allotype and the mec class complex (47).

Hospital-associated MRSA (HA-MRSA)

Beta-lactam resistance among *S. aureus* strains has increased significantly in hospitalized patients, MRSA strains associated with infections in intensive care units, and long hospitalization. In the hospital environment, patients and health care staff are a potential source of MRSA compared to other populations (53,54)**.** HA-MRSA was an actual issue in the nosocomial setting worldwide, and the spread of MRSA among countries was widely recorded (55). these strains are mainly related to bacteremia, urinary tract infections (UTIs), pneumonia, and many acute and chronic infections, Nosocomial MRSA strains are mainly recorded in adult patients, but also in pediatric and neonatal intensive care units (56–58). HA-MRSA infrequently possesses PVL-encoding genes and typically belongs to SCCmec types I, II, and III (59,60). MRSA isolates were marked as HA-MRSA if they were isolated from a sample collected two or more days after a hospital stay; a patient who has had a history of hospital stays, dialysis, surgical intervention, or long-term treatment, throughout the time of the culture; the patient has an indwelling device, or the patient had a previous infection of MRSA, all other isolates of

MRSA are regarded as CA-MRSA (Sato *et al.*, 2017).

Community-associated MRSA (CA-MRSA)

During 1993, the first CA-MRSA originated in Australia, but officially isolated from four American children who died without nosocomial exposure history (62). CA-MRSA strains are genotypically newer and more virulent than HA-MRSA, which emerged during the late 1990s as a significant cause of skin and soft tissue infections in healthy and relatively young individuals without previous hospital exposure (60). CA-MRSA strains are susceptible to many antibiotic families except for β-lactams and cause skin and soft-tissue infections (SSTIs) in 90% of cases. CA-MRSA isolates also show frequent PVL toxin production and mostly carry smaller types of SCCmec (IV, V, and VI) compared to HA-MRSA and exhibit higher fitness, improved capacity to colonize multiple body sites, and are easier to spread (55). Infections with CA-MRSA appear to occur in younger patients and are particularly associated with skin and soft-tissue infections (SSTIs) and toxic shock syndrome. Nevertheless, there are records of serious, life-threatening cases linked to several pathological conditions, such as necrotizing fasciitis and necrotizing pneumonia (63). While it has been predicted that CA-MRSA will replace HA-MRSA in hospitals, statistical models predict coexistence between the two strains given the high discharge and hospitalization levels that improve hospital-community interactions (64). Indeed, CA-MRSA strains already detected in Hospitals (65,66), table 4 summarized the main differences between CA-MRSA and HA-MRSA.

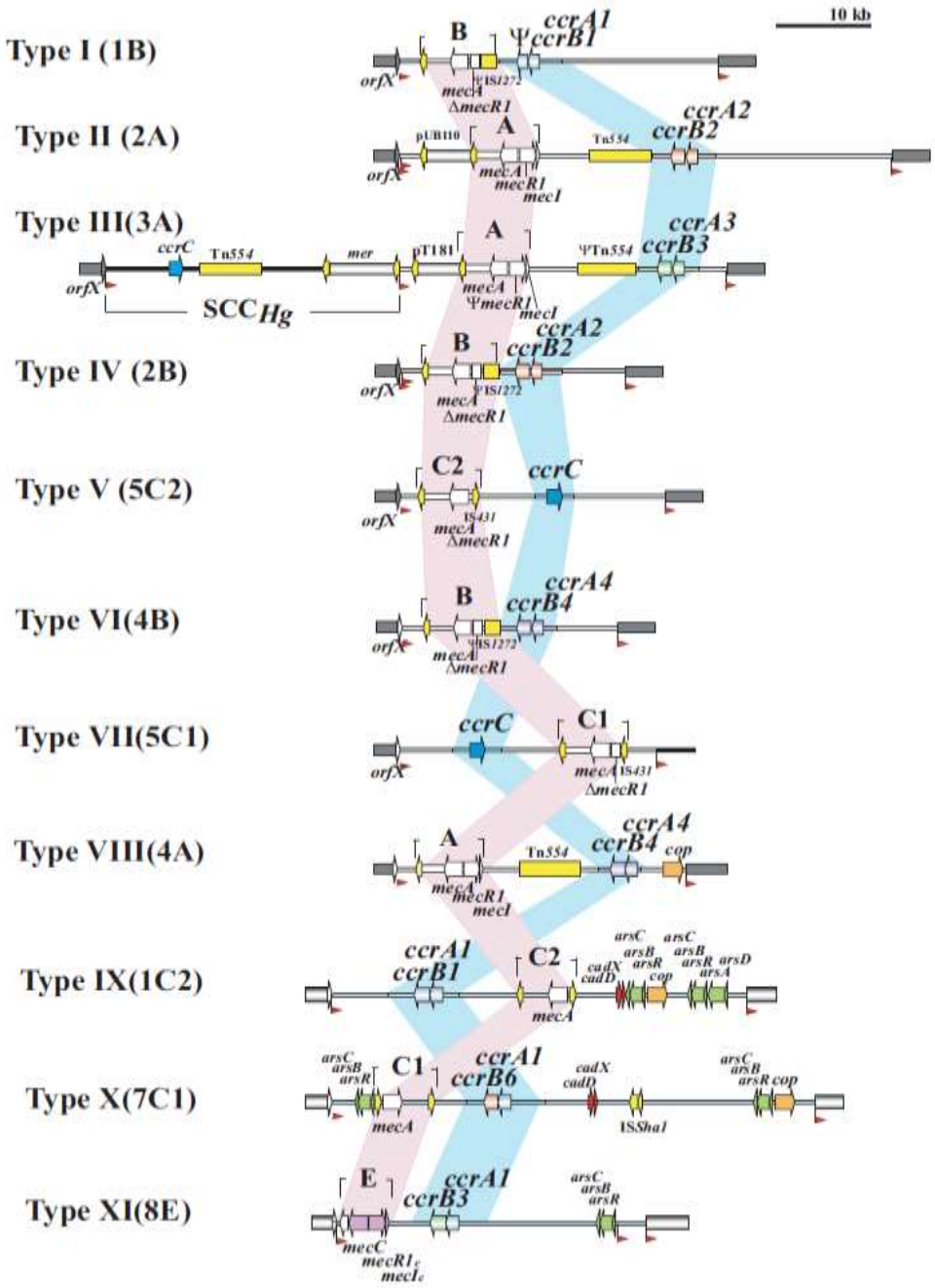

Fig (1): SCCmec elements basic structure (52)

Livestock-Associated MRSA (LA-MRSA)

There have been a few reports of MRSA colonization in livestock animals since the very first evidence of MRSA in mastitis in 1972 (67), recently, MRSA

has been increasingly documented as an emerging problem in veterinary circumference and several strains of MRSA were isolated internationally from cattle, poultry, horses and pigs (68), Besides its association with food-producing animals, it may colonize other host species, and may also cause infections for humans in contact with MRSA colonization animals (61), and has been detected in a variety of food products as well as in clinical cases of livestock animals, including chicken meat, meat products, bovine milk, and bovine mastitis (69). Different clones of LA-MRSA may carry different types of SCCmec elements (IV, V, and XI) in a different country (45,70), furthermore, SCCmec type IX, containing mecC also have been found in this very specific strain (71). However, Sequencing studies have revealed that specific clone of MRSA that cause infections for different animals also may carry the type VIII SCCmec elements (43,72)

In addition, a study conducted by Price et al on the whole-genome sequencing of LA-MRSA isolates from different geographical environments, from humans and livestock, showing that LA-MRSA originated from a human MSSA and jumped to animals (73). There is significant evidence that animals can serve as a reservoir for the emergence of many human pathogenic MRSA clones capable of widespread distribution, as happened 40 years ago with bovine strains of S. aureus (CC97), which jumped from animals to humans and became a human epidemic (Spoor *et al.*, 2013).

Table (4) differences between CA-MRSA and HA-MRSA (75)

Features	CA-MRSA	HA-MRSA
Antibiotic Susceptibility	more Susceptible	Resistant
Spreading	Mostly in younger patients with no previous interaction with health care institutions	Older Patients in regular health-care contact
infections	Skin and soft tissue infections (SSTIs), and necrotizing pneumonia	Septicemia, pneumonia, ventilator-associated pneumonia, surgical site infections
PVL	Positive	Negative
SCCmec types	IV, V, and VI	I, II, and III

Association of integrons type I in *S. aureus*

Horizontal gene transfer (HGT) means the ability to transfer genes from one bacteria to another of the same genus and/or species (76), HGT by antibiotic-resistant determinants carried on the MGEs, such as plasmids, transposons, multidrug resistance genomic islands, and integrons was an essential feature of antimicrobial resistance distribution in bacteria (77). The integrons are a double-stranded DNA capable of acquiring gene cassettes designed to carry genes of drug resistance via a site-specific recombination process (78)**.** All integrons are composed of three main components: (i) The integrase (IntI gene) that plays a significant role in the genetic cassettes recombination (79), (ii) The recombination site (aatI gene) which recognized by the integrase, and conceder a receptor site for gene cassette integration by site-specific

recombination (80,81), (iii) The promoter (Pc gene) which is responsible for the transcription and expression of genetic cassettes within the integron (82). The circular gene cassettes are segments of DNA which can be incorporated by the integrase gene between attI and attC (integron composition described in figure 2-3), This process can also be reversible, which results in the deletion of gene cassettes (83).

S. aureus biofilm formation ability

The Biofilms are multicellular networks of bacteria, where planktonic cells join themselves to strong surfaces (sessile state) and consequently multiply and amass in multilayer cell bunches installed in exceptional three-dimensional structures as mushrooms or towers isolated by fluid-filled channels (84,85). Biofilms are considered as a piece of the typical life pattern of *S. aureus*, the arrangement of biofilms inside host tissues and on embedded clinical devices prompts constant diseases because of their obstinacy to antimicrobial treatments and host immune responses (86). Collecting proof demonstrates that by embracing this way of life, microorganisms in biofilms increase a few points of interest over planktonic cells. For instance, biofilms can shield this organism from the activity of antimicrobial medications, proteases released by host defense cells, and environmental stress factors (87). *S. aureus* biofilm arrangement has been appeared to proceed through five-stage formative steps as follows: attachment, multiplication, exodus, maturation, and spread again (88).

Biofilm comprised of bacterial cells adhere to a layer extracellular polymeric secretions (EPS) which composed of exopolysaccharide, water channels, and environmental DNA (eDNA), that plays an important role in nutrient distribution, its development, and structure constancy, moreover, the presence of diffusive barrier EPS and/or neutralizing enzyme increases the resistance against antimicrobial agents in the biofilm community (89).

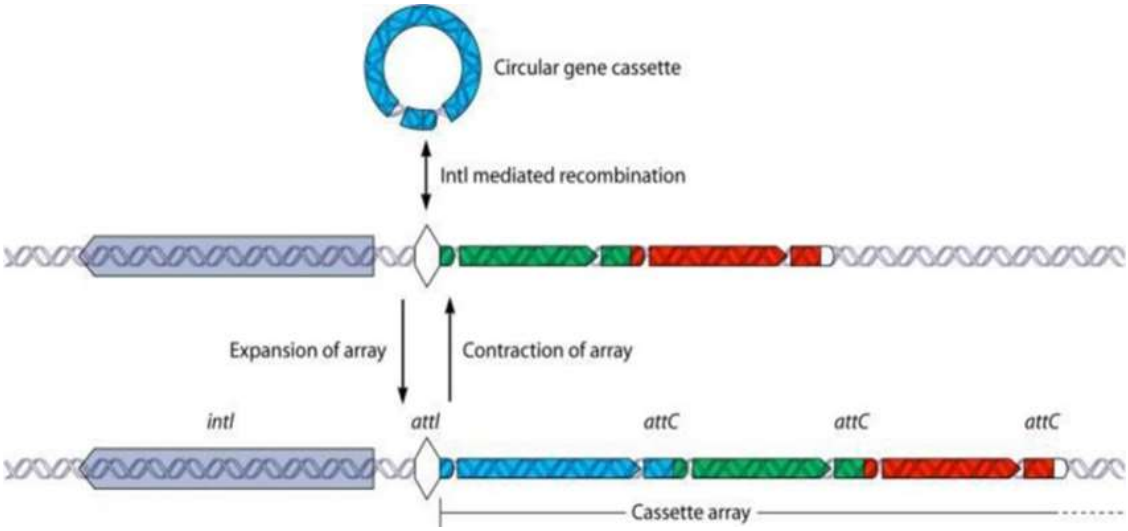

Figure (2-3): Schematic diagram represents integron acquisition of new gene cassettes by site-specific recombination mediated by the integrase protein (81)**.**

References:

1. Ogston A. Micrococcus Poisoning. J Anat Physiol [Internet]. 1882 Oct [cited 2020 May 5];17(Pt 1):24–58. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17231450%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC1310127
2. Ogston A. "On Abscesses." Clin Infect Dis. 1984 Jan 1;6(1):122–8.
3. Licitra G. Etymologia: Staphylococcus . Emerg Infect Dis. 2013 Sep;19(9):1553.
4. Rosenbach FJ. Mikroorganismen bei den Wundinfectionskrankheiten des Menschen. Dtsch Medizinische Wochenschrift. 1884;10(39):631–2.
5. Chapman GH, Berens C, Peters A, Curcio L. Coagulase and Hemolysin Tests as Measures of the Pathogenicity of Staphylococci 1. J Bacteriol. 1934;28(4):343–63.
6. Tan SY, Tatsumura Y. Alexander Fleming (1881–1955): Discoverer of penicillin. Vol. 56, Singapore Medical Journal. Singapore Medical Association; 2015. p. 366–7.
7. Chain E, Florey HW, Gardner AD, Heatley NG, Jennings MA, Orr-Ewing J, et al. PENICILLIN AS A CHEMOTHERAPEUTIC AGENT. Lancet. 1940 Aug 24;236(6104):226–8.
8. Gaynes R. The discovery of penicillin—new insights after more than 75 years of clinical use. Emerg Infect Dis. 2017 May 1;23(5):849–53.
9. William et al. Bergey's Manual of Systematic Bacteriology 2nd edition. Vol. 75, International Journal of Pediatric Otorhinolaryngology. 2009. 392–433 p.
10. Kumar S. Essentials of Microbiology. First. Essentials of Microbiology. Jaypee Brothers Medical Publishers (P) Ltd; 2016.
11. Schleifer K, Bell JA. Rosenbach 1884, 18. 2015.
12. Haag AF, Fitzgerald JR, Penadés JR. Staphylococcus aureus in Animals. Microbiol Spectr. 2019;7(3):1–19.
13. Lan L, Cheng A, Dunman PM, Missiakas D, He C. Golden pigment production and virulence gene expression are affected by metabolisms in Staphylococcus aureus. J Bacteriol. 2010 Jun 15;192(12):3068–77.
14. Talaro KP, Chess B. Foundations in Microbiology, Tenth Edition. 2018. 792,794.
15. harley, J.P . and prescott LM. Laboratory Exercises in Microbiology. Foreign Aff. 2002;
16. Missiakas DM, Schneewind O. Growth and laboratory maintenance of Staphylococcus aureus. Curr Protoc Microbiol. 2013;(SUPPL.28):1–9.
17. Ribeiro de Souza da Cunha M de L. Methods for the Identification, Characterization, and Tracking the Spread of Staphylococcus aureus [Internet]. Staphylococcus aureus. Elsevier Inc.; 2018. 105–125 p. Available from: http://dx.doi.org/10.1016/B978-0-12-809671-0.00006-1
18. Becker K. Pathogenesis of Staphylococcus aureus [Internet]. Staphylococcus aureus. Elsevier

Inc.; 2018. 13–38 p. Available from: http://dx.doi.org/10.1016/B978-0-12-809671-0.00002-4

19. Archer GL. Staphylococcus aureus : A Well–Armed Pathogen . Clin Infect Dis. 1998 May;26(5):1179–81.
20. Roberson JR, Fox LK, Hancock DD, Gay JM, Besser TE. Ecology of Staphylococcus aureus Isolated from Various Sites on Dairy Farms. Vol. 77, Journal of Dairy Science. 1994.
21. NAGASE N, SASAKI A, YAMASHITA K, SHIMIZU A, WAKITA Y, KITAI S, et al. Isolation and Species Distribution of Staphylococci from Animal and Human Skin. J Vet Med Sci [Internet]. 2002 Mar [cited 2020 Jun 22];64(3):245–50. Available from: http://joi.jlc.jst.go.jp/JST.JSTAGE/jvms/64.245?from=CrossRef
22. Peton V, Le Loir Y. Staphylococcus aureus in veterinary medicine. Infect Genet Evol [Internet]. 2014;21:602–15. Available from: http://dx.doi.org/10.1016/j.meegid.2013.08.011
23. Weinert LA, Welch JJ, Suchard MA, Lemey P, Rambaut A, Fitzgerald JR. Molecular dating of human-to-bovid host jumps by Staphylococcus aureus reveals an association with the spread of domestication. Biol Lett [Internet]. 2012 Oct 23 [cited 2020 Jun 23];8(5):829–32. Available from: /pmc/articles/PMC3440972/?report=abstract
24. Foster TJ. Antibiotic resistance in Staphylococcus aureus. Current status and future prospects. FEMS Microbiol Rev. 2017;41(3):430–49.
25. Lowy FD. Antimicrobial resistance: The example of Staphylococcus aureus [Internet]. Vol. 111, Journal of Clinical Investigation. The American Society for Clinical Investigation; 2003 [cited 2020 May 11]. p. 1265–73. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12727914
26. Jevons MP. “Celbenin” -resistant Staphylococci. Br Med J. 1961;1(5219):124–5.
27. Gajdács M. The continuing threat of methicillin-resistant Staphylococcus Aureus. Antibiotics. 2019;8(2).
28. Laurent F, Chardon H, Haenni M, Bes M, Reverdy ME, Madec JY, et al. MRSA harboring mecA variant gene mecC, France. Emerg Infect Dis. 2012 Aug;18(9):1465–7.
29. Harrison EM, Paterson GK, Holden MTG, Larsen J, Stegger M, Larsen AR, et al. Whole genome sequencing identifies zoonotic transmission of MRSA isolates with the novel mecA homologue mecC. EMBO Mol Med. 2013 Apr;5(4):509–15.
30. Petersen A, Stegger M, Heltberg O, Christensen J, Zeuthen A, Knudsen LK, et al. Epidemiology of methicillin-resistant Staphylococcus aureus carrying the novel mecC gene in Denmark corroborates a zoonotic reservoir with transmission to humans. Clin Microbiol Infect [Internet]. 2013 Jan [cited 2020 May

12];19(1):E16–22. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23078039

31. Paterson GK, Morgan FJE, Harrison EM, Peacock SJ, Parkhill J, Zadoks RN, et al. Prevalence and properties of mecC methicillin-resistant Staphylococcus aureus (MRSA) in bovine bulk tank milk in Great Britain. J Antimicrob Chemother [Internet]. 2014 Mar [cited 2020 May 12];69(3):598–602. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24155057
32. Martínez-Meléndez A, Morfín-Otero R, Villarreal-Treviño L, González-González G, Llaca-Díaz J, Rodríguez-Noriega E, et al. Staphylococcal Cassette Chromosome mec (SCCmec) in coagulase negative staphylococci. Med Univ. 2015 Oct 1;17(69):229–33.
33. Moellering RC. MRSA: the first half century. J Antimicrob Chemother. 2012;67(1):4–11.
34. Otter JA, French GL. Molecular epidemiology of community-associated meticillin-resistant Staphylococcus aureus in Europe [Internet]. Vol. 10, The Lancet Infectious Diseases. 2010 [cited 2020 May 14]. p. 227–39. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20334846
35. Shymaa Enany and Laura E. Crotty Alexander. Frontiers in Staphylococcus aureus. Shymaa Enany and Laura E. Crotty Alexander, editor. Vol. 66, Frontiers in Staphylococcus aureus. aneza Trdine 9, 51000 Rijeka, Croatia ©; 2017. 587–599 p.
36. Malachowa N, Deleo FR. Mobile genetic elements of Staphylococcus aureus. Cell Mol Life Sci [Internet]. 2010 Sep [cited 2019 Jul 5];67(18):3057–71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20668911
37. Ito T, Katayama Y, Asada K, Mori N, Tsutsumimoto K, Tiensasitorn C, et al. Structural comparison of three types of staphylococcal cassette chromosome mec integrated in the chromosome in methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother [Internet]. 2001 May [cited 2020 Mar 18];45(5):1323–36. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11302791
38. Shore AC, Deasy EC, Slickers P, Brennan G, O'Connell B, Monecke S, et al. Detection of staphylococcal cassette chromosome mec type XI carrying highly divergent mecA, mecI, mecR1, blaZ, and ccr genes in human clinical isolates of clonal complex 130 methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother. 2011 Aug;55(8):3765–73.
39. IWG-SCC. Classification of staphylococcal cassette chromosome mec (SCCmec): Guidelines for reporting novel SCCmec elements. Vol. 53, Antimicrobial Agents and Chemotherapy. 2009. p. 4961–7.
40. Chongtrakool P, Ito T, Ma XX,

Kondo Y, Trakulsomboon S, Tiensasitorn C, et al. Staphylococcal cassette chromosome mec (SCCmec) typing of methicillin-resistant Staphylococcus aureus strains isolated in 11 Asian countries: A proposal for a new nomenclature for SCCmec elements. Antimicrob Agents Chemother. 2006 Mar;50(3):1001–12.

41. Kaya H, Hasman H, Larsen J, Stegger M, Johannesen TB, Allesøe RL, et al. SCCmecFinder, a Web-Based Tool for Typing of Staphylococcal Cassette Chromosome mec in Staphylococcus aureus Using Whole-Genome Sequence Data. mSphere. 2018;3(1).
42. Zong Z. Characterization of a complex context containing mecA but lacking genes encoding cassette chromosome recombinases in Staphylococcus haemolyticus. BMC Microbiol. 2013;13(1):64.
43. Zhang K, McClure JA, Elsayed S, Conly JM. Novel staphylococcal cassette chromosome mec type, tentatively designated type VIII, harboring class A mec and type 4 ccr gene complexes in a Canadian epidemic strain of methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother [Internet]. 2009 Feb 1 [cited 2019 Sep 14];53(2):531–40. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19064897
44. Wang L, Archer GL. Roles of CcrA and CcrB in excision and integration of staphylococcal cassette chromosome mec, a Staphylococcus aureus genomic island. J Bacteriol. 2010 Jun;192(12):3204–12.
45. Lakhundi S, Zhang K. Methicillin-Resistant Staphylococcus aureus: Molecular Characterization, Evolution, and Epidemiology. Clin Microbiol Rev. 2018;31(4).
46. Liu J, Chen D, Peters BM, Li L, Li B, Xu Z, et al. Staphylococcal chromosomal cassettes mec (SCCmec): A mobile genetic element in methicillin-resistant Staphylococcus aureus. Microb Pathog [Internet]. 2016 Dec 1 [cited 2019 Sep 22];101(July 2018):56–67. Available from: https://www.sciencedirect.com/science/article/pii/S0882401016301760
47. Turlej A, Hryniewicz W, Empel J. Staphylococcal Cassette Chromosome mec (SCCmec) classification and typing methods: An overview. Polish J Microbiol [Internet]. 2011 [cited 2019 Sep 14];60(2):95–103. Available from: https://www.researchgate.net/publication/51637191_Staphylococcal_Cassette_Chromosome_mec_SCCmec_Classification_and_Typing_Methods_an_Overview
48. Deurenberg RH, Vink C, Kalenic S, Friedrich AW, Bruggeman CA, Stobberingh EE. The molecular evolution of methicillin-resistant Staphylococcus aureus [Internet]. Vol. 13, Clinical Microbiology and Infection. Blackwell Publishing Ltd; 2007 [cited 2020 Aug 16]. p. 222–35. Available from: https://pubmed.ncbi.nlm.nih.gov/1

7391376/

49. Van Der Zee A, Heck M, Sterks M, Harpal A, Spalburg E, Kazobagora L, et al. Recognition of SCCmec types according to typing pattern determined by multienzyme multiplex PCR-amplified fragment length polymorphism analysis of methicillin-resistant Staphylococcus aureus. J Clin Microbiol [Internet]. 2005 Dec [cited 2020 Aug 16];43(12):6042–7. Available from: /pmc/articles/PMC1317216/?report=abstract
50. Yang JA, Park DW, Sohn JW, Kim MJ. Novel PCR-restriction fragment length polymorphism analysis for rapid typing of Staphylococcal cassette chromosome mec elements. J Clin Microbiol [Internet]. 2006 Jan 1 [cited 2020 Aug 16];44(1):236–8. Available from: https://europepmc.org/articles/PMC1351960
51. Mehndiratta PL, Bhalla P. Typing of Methicillin resistant Staphylococcus aureus: A technical review. Indian J Med Microbiol. 2012;30(1):16–23.
52. Hiramatsu K, Ito T, Tsubakishita S, Sasaki T, Takeuchi F, Morimoto Y, et al. Genomic basis for methicillin resistance in Staphylococcus aureus. Vol. 45, Infection and Chemotherapy. Infect Chemother; 2013. p. 117–36.
53. Boyce JM. Community-Associated Methicillin-Resistant Staphylococcus aureus as a Cause of Health Care--Associated Infection. Clin Infect Dis. 2008 Mar 15;46(6):795–8.
54. Kallen AJ, Mu Y, Bulens S, Reingold A, Petit S, Gershman K, et al. Health care-associated invasive MRSA infections, 2005-2008. JAMA - J Am Med Assoc. 2010 Aug 11;304(6):641–8.
55. Aires-de-Sousa M. Methicillin-resistant Staphylococcus aureus among animals: current overview. Vol. 23, Clinical Microbiology and Infection. Elsevier B.V.; 2017. p. 373–80.
56. Iwamoto M, Mu Y, Lynfield R, Bulens SN, Nadle J, Aragon D, et al. Trends in invasive methicillin-resistant Staphylococcus aureus infections. Pediatrics. 2013 Oct;132(4).
57. Uhlemann AC, Otto M, Lowy FD, DeLeo FR. Evolution of community- and healthcare-associated methicillin-resistant Staphylococcus aureus. Infect Genet Evol. 2014 Jan;21:563–74.
58. Knox et al. Staphylococcus aureus infections: Transmission within households and the community. Vol. 23, Trends in Microbiology. Elsevier Ltd; 2015. p. 437–44.
59. David MZ, Daum RS. Community-associated methicillin-resistant Staphylococcus aureus: Epidemiology and clinical consequences of an emerging epidemic [Internet]. Vol. 23, Clinical Microbiology Reviews. American Society for Microbiology; 2010 [cited 2020 Mar 24]. p. 616–87. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2901661/pdf/0081-09.pdf

60. Kong et al. Community-Associated Methicillin-Resistant Staphylococcus aureus: An Enemy amidst Us. Vol. 12, PLoS Pathogens. Public Library of Science; 2016.
61. Sato T, Usui M, Konishi N, Kai A, Matsui H, Hanaki H, et al. Closely related methicillin-resistant Staphylococcus aureus isolates from retail meat, cows with mastitis, and humans in Japan. PLoS One. 2017 Oct 1;12(10).
62. CDC. Four pediatric deaths from community-acquired methicillin-resistant staphylococcus aureus - Minnesota and North Dakota, 1997-1999. Conn Med. 1999 Sep 22;63(9):543–5.
63. Bukharie H. A review of community-acquired methicillin-resistant Staphylococcus aureus for primary care physicians. J Fam Community Med. 2010;17(3):117.
64. Kouyos R, Klein E, Grenfell B. Hospital-Community Interactions Foster Coexistence between Methicillin-Resistant Strains of Staphylococcus aureus. Levin BR, editor. PLoS Pathog [Internet]. 2013 Feb 28 [cited 2020 May 21];9(2):e1003134. Available from: https://dx.plos.org/10.1371/journal.ppat.1003134
65. Kateete DP, Namazzi S, Okee M, Okeng A, Baluku H, Musisi NL, et al. High prevalence of Methicillin resistant Staphylococcus aureus in the surgical units of Mulago hospital in Kampala, Uganda. BMC Res Notes. 2011;4.
66. Seni J, Bwanga F, Najjuka CF, Makobore P, Okee M, Mshana SE, et al. Molecular Characterization of Staphylococcus aureus from Patients with Surgical Site Infections at Mulago Hospital in Kampala, Uganda. PLoS One. 2013 Jun 20;8(6).
67. Huber H, Koller S, Giezendanner N, Stephan R, Zweifel C. Prevalence and characteristics of meticillin-resistant staphylococcus aureus in humans in contact with farm animals, in livestock, and in food of animal origin, Switzerland, 2009. Eurosurveillance. 2010 Apr 22;15(16):7–10.
68. Chuang YY, Huang YC. Livestock-associated meticillin-resistant Staphylococcus aureus in Asia: An emerging issue? Vol. 45, International Journal of Antimicrobial Agents. Elsevier B.V.; 2015. p. 334–40.
69. Hata E. Bovine mastitis outbreak in Japan caused by methicillin-resistant Staphylococcus aureus New York/Japan clone. J Vet Diagnostic Investig. 2016;28(3):291–8.
70. Vestergaard M, Cavaco LM, Sirichote P, Unahalekhaka A, Dangsakul W, Svendsen CA, et al. Sccmec type IX element in methicillin resistant Staphylococcus aureus spa type t337 (CC9) isolated from pigs and pork in Thailand. Front Microbiol [Internet]. 2012 Mar 22 [cited 2019 Sep 14];3(MAR):103. Available from: http://journal.frontiersin.org/article/10.3389/fmicb.2012.00103/abstr

act
71. Butaye P, Argudín MA, Smith TC. Livestock-Associated MRSA and Its Current Evolution. Vol. 3, Current Clinical Microbiology Reports. Springer; 2016. p. 19–31.
72. Cavaco LM, Hasman H, Stegger M, Andersen PS, Skov R, Fluit AC, et al. Cloning and occurrence of czrC, a gene conferring cadmium and zinc resistance in methicillin-resistant Staphylococcus aureus CC398 isolates. Antimicrob Agents Chemother. 2010 Sep;54(9):3605–8.
73. Price LB, Stegger M, Hasman H, Aziz M, Larsen J, Andersen PS, et al. Staphylococcus aureus CC398: Host adaptation and emergence of methicillin resistance in livestock. MBio. 2012 Mar 1;3(1):1–6.
74. Spoor LE, McAdam PR, Weinert LA, Rambaut A, Hasman H, Aarestrup FM, et al. Livestock origin for a human pandemic clone of community-associated methicillin-resistant Staphylococcus aureus. MBio. 2013 Aug 13;4(4).
75. Spiliopoulou I, Petinaki E. Methicillin-resistant Staphylococcus aureus colonization and infection risks from companion animals: current perspectives. Vet Med Res Reports. 2015 Nov;6:373.
76. Ochman H, Lawrence JG, Grolsman EA. Lateral gene transfer and the nature of bacterial innovation. Vol. 405, Nature. Nature; 2000. p. 299–304.
77. Deng Y, Wu Y, Jiang L, Tan A, Zhang R, Luo L. Multi-drug resistance mediated by class 1 integrons in Aeromonas isolated from farmed freshwater animals. Front Microbiol. 2016;7(JUN).
78. Akrami F, Rajabnia M, Pournajaf A. Resistance integrons; A mini review. Vol. 10, Caspian Journal of Internal Medicine. Babol University of Medical Sciences; 2019. p. 370–6.
79. Labbate M, Case RJ, Stokes HW. The integron/gene cassette system: an active player in bacterial adaptation. Vol. 532, Methods in molecular biology (Clifton, N.J.). Methods Mol Biol; 2009. p. 103–25.
80. Collis CM, Hall RM. Expression of antibiotic resistance genes in the integrated cassettes of integrons. Antimicrob Agents Chemother. 1995;39(1):155–62.
81. Gillings MR. Integrons: Past, Present, and Future. Microbiol Mol Biol Rev. 2014 Jun 1;78(2):257–77.
82. Boucher Y, Labbate M, Koenig JE, Stokes HW. Integrons: mobilizable platforms that promote genetic diversity in bacteria. Trends Microbiol. 2007 Jul;15(7):301–9.
83. Rowe-Magnus DA, Guérout AM, Mazel D. Super-integrons. Res Microbiol. 1999;150(9–10):641–51.
84. Otto M. Staphylococcal Infections: Mechanisms of Biofilm Maturation and Detachment as Critical Determinants of Pathogenicity. Annu Rev Med. 2013 Jan 14;64(1):175–88.
85. Azara E, Longheu C, Sanna G, Tola S. Biofilm formation and virulence factor analysis of

Staphylococcus aureus isolates collected from ovine mastitis. J Appl Microbiol. 2017 Aug 1;123(2):372–9.

86. Otto M. Staphylococcal biofilms. Vol. 322, Current Topics in Microbiology and Immunology. NIH Public Access; 2008. p. 207–28.
87. Singh R, Ray P, Das A, Sharma M. Penetration of antibiotics through Staphylococcus aureus and Staphylococcus epidermidis biofilms. J Antimicrob Chemother. 2010;65(9):1955–8.
88. Moormeier DE, Bose JL, Horswill AR, Bayles KW. Temporal and stochastic control of staphylococcus aureus biofilm development. MBio. 2014 Oct 14;5(5).
89. Choudhary P, Singh S, Agarwal V. Microbial Biofilms. In: Bacterial Biofilms [Working Title] [Internet]. IntechOpen; 2020 [cited 2020 May 29]. Available from: https://www.intechopen.com/online-first/microbial-biofilms