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Antimicrobial Resistance in Staphylococci at the Human–Animal Interface

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Abstract

The widespread and often indiscriminate use of antimicrobials in animals is considered an important driving force behind the emergence and spread of antimicrobial-resistant bacteria. The emergence of livestock-associated methicillin-resistant *Staphylococcus aureus* and the description of a novel methicillin-resistant gene, *mec*C, have renewed concerns regarding the role of animals as reservoirs and a source for the evolution of novel, virulent zoonotic pathogens. The transfer of antimicrobial-resistant bacteria residing in, or on, animals to close human contacts or the introduction of the bacteria into the food supply chain is a cause for concern. The purpose of this mini-review is to provide a background to the genus *Staphylococcus* and the emergence of antimicrobial resistance as well as a discussion on the most significant antimicrobial resistance mechanisms. The use of antimicrobials in animal husbandry is discussed and the interface between humans and different animal populations is closely examined. Finally, the need for antimicrobial monitoring programmes is discussed and is supplemented with information pertaining to antimicrobial susceptibility testing and molecular typing of staphylococcal isolates.

Keywords: Staphylococci, Antimicrobial Resistance, MRSA, LA-MRSA, Animals

1. Introduction

Staphylococci are natural residents on the skin and mucous membranes of a wide range of host species [1]. Many of the bacterial species have a benign or symbiotic relationship with their host; however, the bacteria may become pathogenic if they gain entry into the host tissue through trauma of the cutaneous barrier [2, 3]. *Staphylococcus aureus* is the most significant species within this genus by virtue of its versatility as a pathogen in humans and animals [4, 5]. In humans, *S. aureus* is responsible for a variety of conditions, ranging from superficial skin infections to life-threatening diseases [6]. In addition, through the production of potent

superantigens and other toxins, *S. aureus* can cause specific toxin-mediated conditions such as toxic shock syndrome, scalded skin syndrome and food poisoning [6]. In animals, *S. aureus* is a common cause of intramammary infections (IMIs), or mastitis [7]. Worldwide, the dairy industry incurs significant financial losses annually due to intramammary infections [8–10].

Other *Staphylococcus* species, collectively termed coagulase-negative staphylococci (CNS), are responsible for a variety of opportunistic infections in humans and animals [11]. Due to the ubiquity of many of the species within this group, their clinical significance has traditionally been dismissed, and when isolated from clinical specimens, the bacteria have merely been regarded as contaminants [12]. This perception is, however, changing as many species have emerged as important causes of nosocomial infections, particularly in relation to foreign-device-related infections and infections in immunocompromised patients [1, 13].

The propensity for staphylococci to develop antimicrobial resistance is a cause for great concern in both human and veterinary medicine [14]. As the efficacy of antimicrobials declines, the morbidity and mortality in infected patients increase [15, 16]. Moreover, in the case of human medicine, the costs associated with the treatment of infections caused by antimicrobial-resistant bacteria represent a serious public health burden in hospital and community settings [10].

2. The genus *Staphylococcus*

2.1. Classification of staphylococci

Before the 1970s, *S. aureus* and *S. epidermidis*, or *S. albus* as it was originally named, were the only recognized *Staphylococcus* species [17]. *Staphylococcus aureus* was considered a pathogen and *S. epidermidis*, when isolated from clinical material, was regarded as a contaminant [17]. In the mid-1970s, Kloos and Schleifer [17–19] conducted comprehensive systematic studies of staphylococci and micrococci and described a number of new species. To date, 49 species and 26 subspecies have been described and with improvements in the accuracy of genotyping methods the number of species is still increasing [20, 21].

The genus *Staphylococcus* is classified along with the genera *Jeotgalicoccus*, *Macrococcus*, *Nosocomiicoccus* and *Salinicoccus* in the family *Staphylococcaceae* [12, 21]. The full Linnaean classification for the genus and the type species, *S. aureus*, is shown in Table 1.

In diagnostic laboratories, staphylococci are historically differentiated by their ability to produce the enzyme coagulase, which mediates the conversion of fibrinogen to fibrin resulting in the clotting of blood [22]. The production of coagulase has long been recognized as an important indicator of pathogenicity [23, 24], and the coagulation of rabbit plasma provides a rapid *in vitro* method for differentiating pathogenic coagulase-positive staphylococci (CPS) and 'non-pathogenic' coagulase-negative staphylococci [1, 24].

Seven CPS are currently recognized, namely *S. aureus*, *S. lutrae*, *S. schleiferi* subsp. *coagulans*, the coagulase-variable, *S. hyicus* and the *S. intermedius* group (SIG), which comprises *S.*

Taxonomy	Name
Domain	*Bacteria*
Kingdom	*Eubacteria*
Phylum	*Firmicutes*
Class	*Bacilli*
Order	*Bacillales*
Family	*Staphylococcaceae*
Genus	*Jeotgalicococcus*
	Macrococcus
	Nosocomiicoccus
	Salinococcus
	Staphylococcus
Species	*Staphylococcus aureus*
Subspecies	*Staphylococcus aureus* subsp. *aureus*
	Staphylococcus aureus subsp. *anaerobius*

Table 1. The current Linnaean classification scheme for the genus *Staphylococcus* [21].

intermedius, *S. pseudintermedius* and *S. delphini* [25, 26]. *Staphylococcus aureus*, which is known to be pathogenic in both humans and animals, is considered to be the most important of all the CPS. Other CPS, particularly *S. hyicus* and members of the SIG group, are important veterinary pathogens and are responsible for infections in a number of different animal species [2, 25, 26].

The CNS comprise a biochemically heterogeneous group of bacteria which have, for convenience, been grouped together by virtue of their inability to produce the enzyme coagulase [23, 24]. The susceptibility of CNS isolates to novobiocin has been shown to be a useful phenotypic characteristic in diagnostic laboratories to differentiate *S. saprophyticus* from other clinically important species [2, 27]. The phylogenetic relationship between the coagulase-negative staphylococcal species has recently been clarified through the analysis of four gene loci, namely the 16S rRNA gene and the three protein-encoding genes, *dna*J, *rpo*B and *tuf*, which code for heat shock protein 40, the β-subunit of RNA polymerase and elongation factor Tu, respectively [12, 28]. The molecular analysis resolved the CNS into 14 cluster groups, which are depicted in Figure 1.

2.2. General characteristics of staphylococci

Staphylococci are non-motile, non-sporeforming Gram-positive coccus-shaped bacteria [29]. The cocci may occur singly, in pairs and in tetrads, and they characteristically divide in more than one plane to form irregular 'grape-like' clusters [2, 29]. In fact, the name *Staphylococcus* is derived from the Greek words '*staphyle*' and '*kokkos*' meaning 'bunch of grapes' and 'berry', respectively [1, 29]. Most staphylococci are facultative anaerobes and catalase positive with

NOVOBIOCIN-SUSCEPTIBLE STAPHYLOCOCCI

Auricularis
S. auricularis

Muscae
S. muscae
S. microti
S. rostri

Hyicus
S. hyicus
S. agnetis
S. chromogenes
S. felis

Intermedius
S. intermedius
S. delphini
S. lutrae
S. pseudintermedius
S. schleiferi subsp. schleiferi
S. schleiferi subsp. coagulans

Aureus
S. aureus subsp. aureus
S. aureus subsp. anaerobius
S. simiae

Epidermidis
S. epidermidis
S. capitis subsp. capitis
S. capitis subsp. urealyticus
S. caprae
S. saccharolyticus

Warneri
S. warneri
S. pasteuri

Haemolyticus
S. haemolyticus
S. devriesei
S. hominis subsp. hominis
S. hominis subsp. novobiosepticus
S. jettensis
S. petrasii subsp. croceilyticus
S. petrasii subsp. petrasii

Lugdunensis
S. lugdunensis

Pettenkoferi-Massiliensis
S. pettenkoferi
S. massiliensis

Simulans-Carnosus
S. simulans
S. carnosus subsp. carnosus
S. carnosus subsp. utilis
S. condimenti
S. piscifermentans

NOVOBIOCIN-RESISTANT STAPHYLOCOCCI

Saprophyticus
S. saprophyticus subsp. saprophyticus
S. saprophyticus subsp. bovis
S. equorum subsp. equorum
S. equorum subsp. linens
S. gallinarum
S. succinus subsp. succinus
S. succinus subsp. casei
S. xylosus

Cohnii-Nepalensis
S. cohnii subsp. cohnii
S. cohnii subsp. urealyticus
S. nepalensis

Arlettae-Kloosii
S. arlettae
S. kloosii

Sciuri
S. sciuri subsp. sciuri
S. sciuri subsp. carnaticus
S. sciuri subsp. rodentium
S. fleurettii
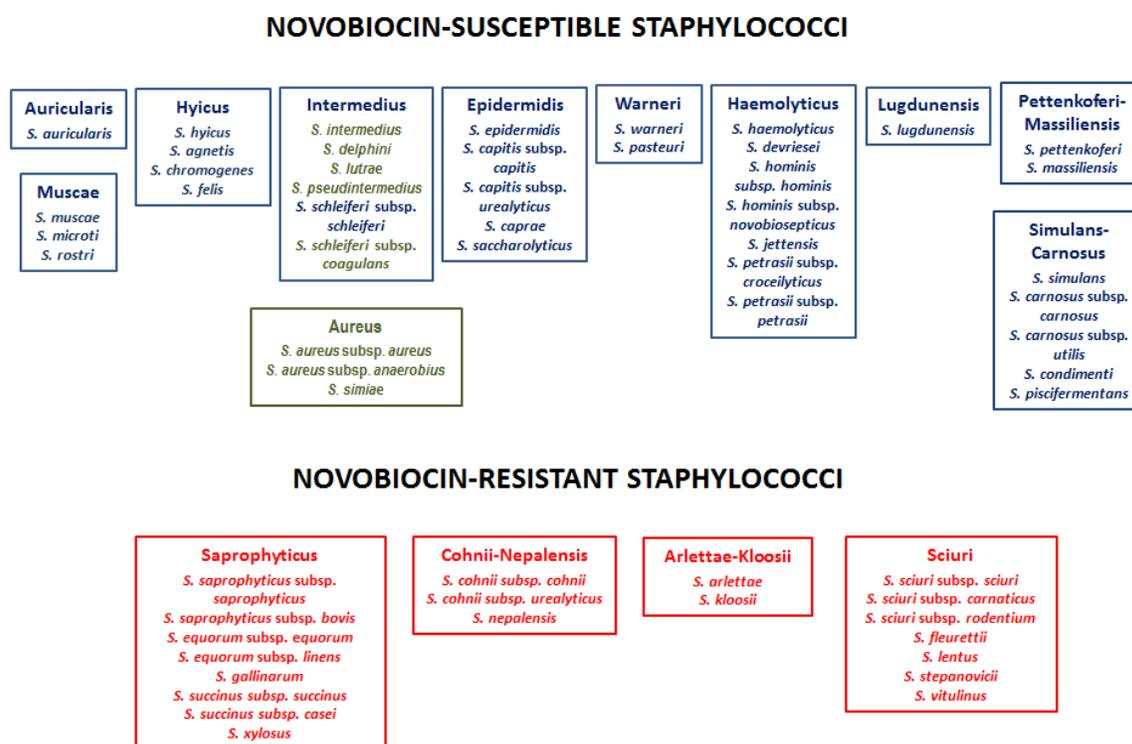S. lentus
S. stepanovicii
S. vitulinus

Figure 1. Phylogenetic separation of staphylococcal species and subspecies. Coagulase-positive *Staphylococcus* spp. are shown in green font [12, 28].

the exception of *S. aureus* subsp. *anaerobius* and *S. saccharolyticus* [1]. Staphylococci can grow in a wide pH range (4.8–9.4) and can survive temperatures of up to 60°C for 30 minutes [29]. Many *Staphylococcus* species are tolerant of high salt concentrations (7.5–10%) due to the production of osmoprotectants [29]. The ability to grow in the presence of above-average salt concentrations explains the predilection of many staphylococcal species for the sebaceous surfaces of mammals [1]. This phenotypic trait is exploited in diagnostic laboratories by incorporating high concentrations of sodium chloride into agar media to selectively isolate staphylococci from contaminated samples [1, 5].

Staphylococcus aureus is able to exist as a commensal on the skin and mucous membranes of different hosts, but when the opportunity presents, the bacterium is able to become pathogenic [1]. *Staphylococcus aureus* can colonize a number of sites on the human body with the anterior nares being the preferred site [30, 31]. Approximately 20% of healthy humans are persistent nasal carriers of *S. aureus*, about 30% are intermittent carriers and around 50% of individuals are never colonized with *S. aureus* [31, 32]. Individuals who are colonized by *S. aureus* are at a higher risk of becoming infected and are also an important source for the dissemination of *S. aureus* among individuals in the community [1, 33]. The primary means of transmission of *S. aureus* is by direct contact, usually skin-to-skin contact with colonized or infected individuals, although indirect means, via fomites, is also thought to play a role [33]. Various host factors, including loss of the normal skin barrier, the presence of underlying diseases, such as diabetes and acquired immunodeficiency syndrome, predispose individuals to infection [33].

The success of *S. aureus* as a pathogen is attributed in part to the capacity of the bacteria to produce a diverse array of virulence factors [1, 14]. Some of these factors may be more important than others in different diseases or at different stages of pathogenesis as not all factors are produced by each strain [34, 35]. Based on structure and functionality, the virulence factors can be broadly divided into two general groups, namely surface-associated factors and degradative enzymes, including exotoxins [36]. The microbial surface components of *S. aureus* recognizing the adhesive matrix molecular components (MSCRAMMs) comprise surface proteins that promote colonization by binding to host cells [36]. This group, which includes fibrinogen-, fibronetin- and collagen-binding proteins, is important during the initial stage of infection [37]. Once infection is established, the expression of tissue-binding proteins is downregulated, whilst the synthesis of extracellular toxins and tissue-degrading enzymes is induced to aid the acquisition of nutrients and the dissemination of the bacteria [38].

The CNS constitute a significant proportion of the natural microflora colonizing the skin and mucous membranes of humans and animals [12, 39]. The different staphylococcal species display apparent site or niche preferences on their hosts and occur more frequently at these sites [2, 12]. *Staphylococcus epidermidis* is the most abundant and widely distributed species on human skin and can occur in densities of 10^3 to 10^4 cells cm^{-2} [12, 40, 41]. *Staphylococcus epidermidis* is particularly prevalent in moist areas, such as the axillae, inguinal and perineal areas, anterior nares, conjunctiva and toe webs [12]. *Staphylococcus haemolyticus* and *S. hominis* are preferentially isolated from areas of the skin where there are numerous apocrine glands such as the axillae and pubic areas, whereas *S. capitis* is typically located around the sebaceous glands on the forehead and scalp following puberty [2, 12]. *Staphylococcus warneri* is commonly recovered from human hands, whilst *S. lugdunensis* has a preference for the inguinal and breast areas [41–43].

Coagulase-negative staphylococci are typically less pathogenic than *S. aureus* possessing a smaller array of virulence factors [12]. However, CNS often exhibit greater resistance to antimicrobials and also have a greater tendency to develop multidrug resistance [44]. Coagulase-negative staphylococci are believed to serve as reservoirs of antimicrobial resistance genes, which can transfer and integrate into the *S. aureus* genome leading to the emergence of new, potentially more resistant strains [45, 46].

3. Genomic organization and genetic flexibility of *S. aureus*

The staphylococcal genome consists of a closed circular molecule of double-stranded DNA between two and three megabase pairs in length and encoding between 2 509 and 2 892 openread frames [1, 47]. Whole genome sequencing of a number of *S. aureus* strains has revealed that approximately 75% of the bacterium's genome comprises a core component, common to all strains [6]. The majority of the genes comprising the core genome are those associated with central metabolism and other housekeeping functions [48]. The remaining 25% of the *S. aureus* genome, termed the accessory genome, contains genes that encode a diverse array of non-essential functions ranging from virulence, antimicrobial and metal resistance, to sub-

strate utilization and miscellaneous metabolism [49]. Many of the regions making up the accessory genome are, or once were, mobile genetic elements (MGEs), such as chromosomal cassettes, pathogenicity islands, plasmids, prophages and transposons [50]. Mobile genetic elements can be transferred horizontally between bacteria of the same or different species, leading to the evolution of bacterial strains [50, 51]. The distribution of these elements is therefore important from a clinical perspective, as it may lead to the evolution of bacterial strains that are potentially more virulent or resistant to antimicrobials [50].

3.1. Host specificity and host switching of *S. aureus*

Devriese and Oeding [52] were amongst the first researchers to note the occurrence of phenotypic differences between *S. aureus* strains isolated from humans and different animal hosts. A simplified biotyping scheme was developed by Devriese and co-workers to differentiate *S. aureus* isolates into ecological variants, or ecovars, that delineated along human, poultry or ruminant associations [53, 54]. Many strains, however, were found not to belong to any of the host-specific biotypes and instead were classed as non-host-specific biotypes which are usually associated with several hosts [55]. The use of phenotyping techniques such as multilocus enzyme electrophoresis (MLEE) [56] and later more discriminatory genotyping methods, such as pulsed-field gel electrophoresis (PFGE) [55, 57], multilocus sequence typing (MLST) [56, 58] and whole genome sequencing [59], has clearly demonstrated the existence of specialized host-specific *S. aureus* clones [54].

Microarray studies of animal and human *S. aureus* isolates have shown that strains that are isolated from one host species tend to be uncommon in other species [60], although this delineation is not always absolute [54]. In many respects, the host range of *S. aureus* should be considered an evolving trait [61]. Adaptation to a particular host species does not prevent *S. aureus* strains from causing occasional infections in other species [62]. Wherever there is an interface between different host species, the opportunity exists for bacterial exchange. In most cases, these exchanges lead to transient infections which are short lived due to the failure of the *S. aureus* strain to establish transmission pathways in the new host species [62]. However, sustained interspecies events are known to occur albeit at a lower frequency [62].

A number of independent studies have investigated specific *S. aureus* host-switching events. All of the described host-switch events highlight the significant role that the transfer of MGEs plays in host adaptation and specialization [56, 62, 63]. It is believed that if the conditions under which *S. aureus* host switches occur is understood, then strategies could be developed to curb future host jumps and the emergence of new human pathogens [63].

4. Staphylococcal infections in humans

Infections caused by *S. aureus* are often acute and pyogenic and, if left untreated, may spread to surrounding tissue or via bacteremia to metastatic sites [2]. Some of the most common infections caused by *S. aureus* involve the skin, and include furuncles or boils, cellulitis, impetigo and post-operative wound infections of various sites [2]. Mastitis is one of a variety

of skin and soft tissue infections that may be caused by *S. aureus*. Unlike other *S. aureus* infections in humans, staphylococcal mastitis has not been extensively studied [60, 64]. It is estimated that mastitis develops in approximately 1–3% of nursing mothers [65]. Infection usually presents within two to three days after giving birth, with symptoms ranging from cellulitis to abscess formation [65]. In severe cases, systemic symptoms such as fever and chills may arise [65]. *Staphylococcus aureus* may also cause more serious infections such as bacteremia, pneumonia, osteomyelitis, acute endocarditis, myocarditis, pericarditis, cerebritis, meningitis and abscesses of the muscle, urogenital tract, central nervous system and various intra-abdominal organs [2].

Staphylococcal diseases that arise exclusively from the production of staphylococcal toxins include staphylococcal scalded skin syndrome (SSSS), toxic shock syndrome (TSS) and staphylococcal food poisoning [65]. Staphylococcal food poisoning occurs following the ingestion of food contaminated with enterotoxins [66]. Enterotoxins are heat stable and can survive conditions that would ordinarily kill bacteria [67]. Furthermore, enterotoxins are tolerant to low pH conditions and the activity of proteolytic enzymes and are thus able to retain their activity in the digestive tract following ingestion [5, 67]. Following ingestion of contaminated food and a short incubation period (two to eight hours), nausea and vomiting ensue [66]. Diarrhea, hypotension and dehydration may also occur [65]. Staphylococcal food poisoning is usually self-limiting and typically resolves within 24 to 48 hours following the onset of symptoms [3]. Occasionally, the symptoms may be severe enough to warrant hospitalization, particularly in the case of infants, the elderly or immunocompromised individuals [66]. Staphylococcal food poisoning is a common disease but the true incidence is considered to be underestimated due to misdiagnosis, unreported outbreaks, improper specimen collection and laboratory examination [66]. The disease represents a considerable burden in terms of loss of productivity, medical and hospital expenses and financial losses to food industries [66]. Enterotoxin production is not limited to *S. aureus* but has been documented in a number of other staphylococci including *S. hyicus*, *S. pseudintermedius*, *S. chromogenes*, *S. cohnii*, *S. epidermidis*, *S. lentus*, *S. lugdunensis*, *S. saprophyticus*, *S. sciuri*, *S. warneri* and *S. xylosus* [3, 5, 68, 69].

Almost half of all the CNS species that have been identified to date have been implicated in human infections [65]. Coagulase-negative staphylococci, in particular *S. epidermidis*, are frequently responsible for nosocomial infections and prosthetic-device-related infections [27, 70]. The increased infection rate is correlated with increase in the use of prosthetic and indwelling devices in hospitals as well as the larger number of immunocompromised patients [39, 41]. *Staphylococcus epidermidis* is uniquely adapted to colonize prosthetic devices by virtue of the ability of the bacterium to produce an extracellular polysaccharide, also referred to as a glycocalyx or slime layer, which facilitates the formation of a protective biofilm on the surface of the implanted device [39, 65]. The process of biofilm formation and the protective effects conferred upon the bacteria are discussed in further detail below.

Staphylococcus haemolyticus is the second most frequently encountered CNS associated with human infections [2]. *Staphylococcus haemolyticus* has been implicated in native valve endocarditis, septicemia, peritonitis, urinary tract infections and wound and bone and joint infections [2]. *Staphylococcus saprophyticus* is another opportunistic pathogen, which is frequently

responsible for causing human urinary tract infections, particularly in young, sexually active females [2, 12].

Two staphylococcal species, *S. lugdunensis* and *S. schleferi*, have been described as emerging zoonotic pathogens [71]. *Staphylococcus lugdunensis*, which is known to cause skin infections and invasive infections, such as endocarditis, osteomyelitis and sepsis in humans, has more recently been described as an animal pathogen implicated in respiratory and skin infections [71, 72]. *Staphylococcus schleiferi*, which has typically been associated with skin infections in pet animals, has also been found associated with endocarditis and metastatic infection as well as endophthalmitis in humans [73, 74]. Both bacterial species have been reported to cause more serious infections than other CNS, but the exact reasons for this enhanced virulence are not known [43, 71].

5. Staphylococcal infections in animals

Amongst all of the described staphylococcal species, only *S. aureus*, *S. epidermidis*, *S. hyicus* and *S. pseudintermedius* are responsible for significant disease conditions in animals [75, 76]. Other *Staphylococcus* spp. are predominantly associated with opportunistic infections in different animal species [75].

In poultry, *S. aureus* is responsible for several infectious conditions including septic arthritis, subdermal abscesses ('bumblefoot'), gangrenous dermatitis and bacterial chrondronecrosis with osteomyelitis [58, 77]. In sheep and goats, *S. aureus* is a common cause of dermatitis whilst in horses and pigs *S. aureus* may cause botryomycosis, a chronic, suppurative granulomatous condition [24]. In companion animals, *S. aureus* causes suppurative conditions similar to those produced by *S. pseudintermedius* [24].

Staphylococcus hyicus is responsible for causing exudative epidermitis in pigs, also known as greasy pig disease, as well as sporadic joint infections and cystitis [24]. In companion animals *S. pseudintermedius* is commonly isolated from cases of pyoderma, otitis externa and other suppurative conditions including mastitis, endometritis, cystitis, osteomyelitis and wound infections [24]. Methicillin-resistant *S. pseudintermedius* is emerging as an important clinical problem in veterinary medicine in many countries [78, 79].

Staphylococcus species can cause intramammary infections in a variety of animal species [24]. Bovine IMIs are the most economically significant, but in areas where sheep and goats are maintained for milking purposes, IMIs caused by staphylococci can cause substantial losses [80]. Similarly, in countries where milk is sourced from buffalo or camels, significant financial losses due to mastitis have been reported [81, 82]. The direct, or obvious, financial losses incurred as a result of IMIs include treatment costs (veterinary fees and drugs); milk that is discarded due to poor quality, or milk lost during the required withdrawal period before and after drug administration; increased labor costs and animal fatalities or euthanasia [83, 84]. In addition to the direct financial losses incurred due to IMIs, a number of indirect costs exist, which are harder to quantify and are often overlooked. Subclinical infections usually proceed

undetected in a herd resulting in a gradual decrease in milk production and a decline in overall milk quality [83]. This leads to a gradual erosion of profit margins, which, even when detected, can take significant time and financial input to rectify [83].

Staphylococcus aureus is possibly the most notorious of all mastitis pathogens by virtue of the fact that infections caused by this species are difficult to treat and tend to become chronic [36]. Coagulase-negative staphylococci are considered to be emerging pathogens, as in many countries the CNS have become the most common bacteria isolated from intramammary infections [9]. The species most commonly isolated from intramammary infections include *S. chromogenes*, *S. epidermidis*, *S. haemolyticus*, *S. simulans* and *S. xylosus* [85, 86].

6. Antimicrobial resistance in staphylococci

Staphylococcus aureus is intrinsically susceptible to all antimicrobials that have been developed [33]. Antimicrobial resistance may be acquired through mutation and selection of resistant bacterial strains or through horizontal transfer of resistance genes from other bacteria of the same or different species [33]. Common mechanisms which are used to circumvent the action of antimicrobials include (i) the production of enzymes that inactivate or destroy the antimicrobial; (ii) a reduction of the bacterial cell wall permeability limiting the antimicrobial access into the cell; (iii) the development of alternative metabolic pathways to those inhibited by the antimicrobial; and (iv) active elimination of the antimicrobial from the bacterial cell or the target site [87, 88]. The mechanisms responsible for antimicrobial resistance in CNS are identical to those occurring in *S. aureus* [89].

6.1. The emergence of resistance in *S. aureus*

Shortly after the introduction of penicillin in human medicine in 1946, reports of *S. aureus* strains exhibiting resistance to this antimicrobial began emerging [90]. Penicillin-resistant staphylococci were first recognized in hospitals and then subsequently in the community [91]. By the late 1960s, more than 80% of both community- and hospital-associated staphylococcal isolates were resistant to penicillin [92]. It is estimated that more than 90% of staphylococcal isolates now produce penicillinase, regardless of the clinical setting [93].

A similar clinical scenario was observed following the introduction of methicillin, the first semisynthetic penicillin resistant to the action of penicillinase [90]. Shortly after the introduction of methicillin in 1959, methicillin-resistant strains were reported [94]. Once again, resistant strains initially presented in the hospital environment; and then by the late 1990s, virulent methicillin-resistant clones emerged in the community [91].

During the 1960s, a number of non-β-lactam antibiotics, such as chloramphenicol, erythromycin, streptomycin and tetracycline, were introduced [89]. Although initially effective against *S. aureus*, resistance to these antimicrobials was eventually observed [89]. By 1976, resistance to gentamicin and kanamycin had been reported, and by the early 1980s, multidrug-resistant *S. aureus* strains were reportedly responsible for nosocomial outbreaks in many countries [47, 95].

Vancomycin and teicoplanin, both glycopeptide antibiotics, have been the frontline treatment for serious methicillin-resistant *Staphylococcus aureus* (MRSA) infections for the last 15 years [47, 96]. Due to the increasing burden of MRSA infections and the concomitant increase in the usage of vancomycin, bacterial isolates showing intermediate susceptibility (not inhibited *in vitro* at concentrations below 4–8 µg/ml, vancomycin-intermediate *S. aureus* (VISA)) were reported in Japan in 1997 [97]. By 2002, vancomycin-resistant *S. aureus* (VRSA; isolates only inhibited at antimicrobial concentrations of 16 µg/ml or more) were encountered in Michigan, United States [33, 98].

A timeline showing the emergence of resistance in *S. aureus* relative to the introduction of significant antimicrobial classes is shown in Figure 2. Several antimicrobials with good anti-staphylococcal activity have been introduced in recent years, including ceftaroline, ceftobi-prole, dalbavancin, daptomycin, linezolid, telavancin and tigecycline [99, 100]. Isolates showing reduced susceptibility to daptomycin and resistance to linezolid have already been documented [101]. Undoubtedly, as the use of these drugs becomes more widespread, bacterial resistance will become more common [102].
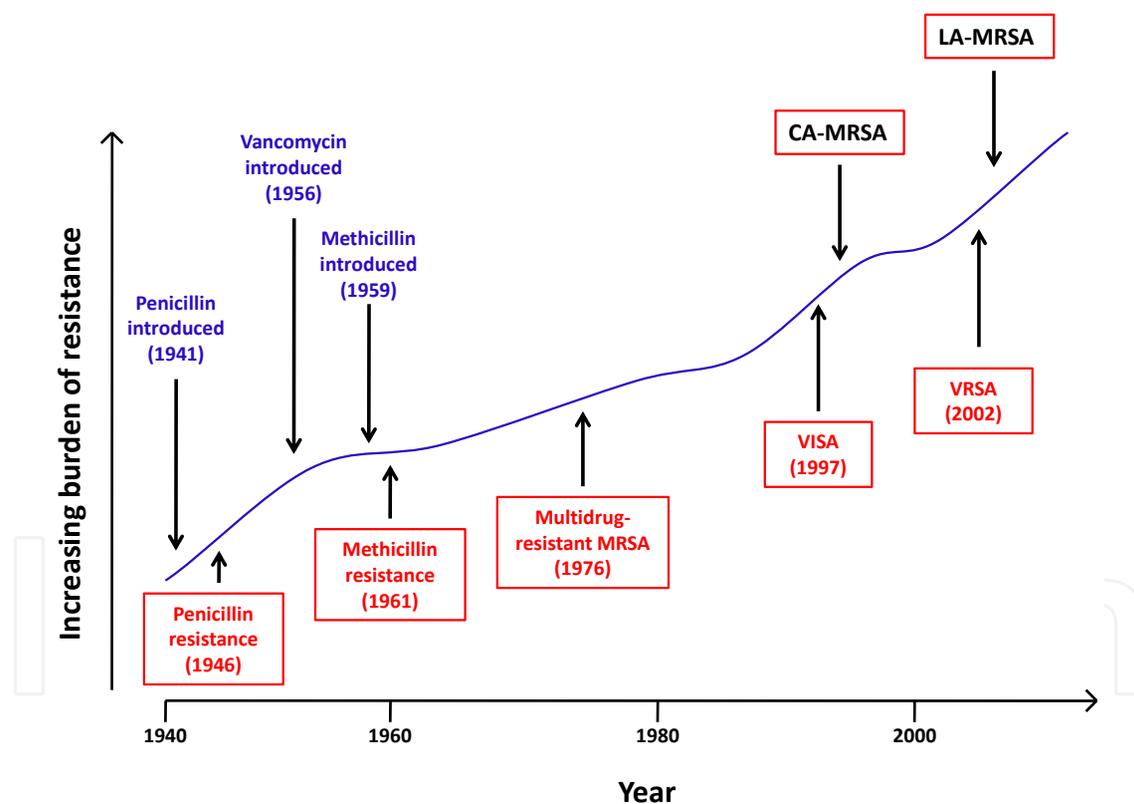

Figure 2. The emergence of antibiotic resistance in *S. aureus* (VISA, vancomycin-intermediate *S. aureus*; VRSA, vancomycin-resistant *S. aureus*; CA-MRSA, community-associated methicillin-resistant *S. aureus*; LA-MRSA, livestock-associated methicillin-resistant *S. aureus*) Adapted from [33, 47].

The distinct lack of novel antimicrobials for future use is a serious cause for concern [93, 103]. Current strategies are aimed at prudent and strategic use of antimicrobials to delay the emergence of resistance and ensure the longevity of antimicrobials in clinical practice [104, 105].

6.2. Mechanism of penicillin resistance in staphylococci

Resistance to penicillin is primarily mediated by the *bla*Z gene, which is responsible for the production of beta-lactamase (penicillinase), an enzyme that hydrolyzes the β-lactam ring of the penicillin molecule [93]. The *bla*Z gene is part of a transposable element located on a large plasmid, which often carries additional antimicrobial resistance genes, which confer resistance to erythromycin, fusidic acid and gentamicin [93]. The plasmid may also carry genes encoding resistance to disinfectants (quaternary ammonium compounds), dyes (acriflavine and ethidium bromide) or heavy metals (cadmium, lead and mercury) [106].

6.3. Mechanism of methicillin resistance in staphylococci

Methicillin resistance arises due to the acquisition of the *mec*A gene, which encodes an alternative penicillin-binding protein, PBP2a (or PBP2'), which has a low affinity for β-lactam antibiotics [14, 100, 107]. The synthesis of PBP2a allows bacterial cell cell wall synthesis to proceed uninterrupted in the presence of β-lactam antibiotics despite the inactivation of the native penicillin-binding protein of the cells [93, 100]. The *mec*A gene confers resistance to all β-lactam antibiotics, including cephalosporins, cefamycins and carbapenems [103, 107].

The *mec*A gene is part of a large mobile genetic element designated the staphylococcal cassette chromosome mec (SCC*mec*) [31, 100]. The SCC*mec* integrates into the staphylococcal chromosome of methicillin-sensitive *S. aureus* at a specific site (*att*B*scc*) which is located at the 3' end of an open reading frame of a gene with an unknown function (*orfX*) [33, 108]. In addition to the *mec*A gene, SCC*mec* also carries the genes that control the transcription of the *mec*A gene (*mec*I and *mec*R1) and chromosomal cassette recombinase genes (*ccr*A, *ccr*B or *ccr*C), which mediate the integration and excision of the cassette into the host chromosome [31]. The SCC*mec* element may also contain other genes encoding resistance to antimicrobials, such as aminoglycosides or macrolides and resistance to heavy metal ions [109, 110]. According to their genetic structure and contents, SCC*mec* elements are categorized into several types and subtypes [14, 31]. To date, the website of the International Working Group on the Classification of Staphylococcal Cassette Chromosome elements (IWCC) lists 11 types of SCC*mec* elements (I to XI) [111].

Staphylococcal chromosomal cassettes containing the *mec* gene have been identified not only in *S. aureus* but also in other CPS and CNS [112]. In CNS, SCC*mec* elements exhibit a more polymorphous structure with a larger number of *ccr*–*mec* combinations being encountered, which have not been described for MRSA [113]. The higher frequency and diversity of SCC*mec* elements in CNS suggest that CNS are a potential reservoir of SCC*mec* elements, which may facilitate and drive the emergence of new MRSA clones [114]. The possible mechanism(s) involved in the horizontal transfer of SCC*mec* elements from CNS to *S. aureus* are currently not known [115].

The origin of the *mec*A gene has been a source of speculation for many years. Homologues of the *mec*A gene have been found in *S. sciuri* and *S. vitulinus,* but in both cases, the *mec*A gene is not located in a *mec*A complex as with SCC*mec* [116]. Tsubakishita and co-workers [108]

identified a *mec*A gene homologue in *S. fleuretti*, which shared almost 100% sequence homology with MRSA strain N315 and which resided on a structure almost identical to the *mec*A complex. *Staphylococcus fleuretti* is a member of the *S. sciuri* group of staphylococci and is a commensal bacterium of animals [108]. The occurrence of a direct precursor of the methicillin resistance determinant in a *Staphylococcus* species, which normally resides on animals, suggests that staphylococci of animal origin may be a reservoir for the evolution of novel SCC*mec* elements [116].

Molecular investigations of a *S. aureus* isolate, which was found to be phenotypically resistant to methicillin but negative for the *mec*A gene when tested with a standard diagnostic polymerase chain reaction (PCR) assay, led to the discovery of a novel *mec*A homologue [117]. The *mec*A homologue, initially designated $mec\text{A}_{LGA251}$ after *S. aureus* LGA251, the bacterial strain in which the gene was first sequenced, shares 70% nucleotide identity with the conventional *mec*A gene [118]. The work of García-Álvarez and co-workers [117] showed that $mec\text{A}_{LGA251}$ was found in *S. aureus* lineages typically associated with cattle, namely clonal complex (CC)130, CC1943 and sequence type (ST)425, suggesting the existence of a zoonotic MRSA reservoir. Furthermore, evidence of animal-to-human transmission of MRSA strains harboring $mec\text{A}_{LGA251}$ has been documented [119]. In 2012, the IWCC renamed the *mec*A variant, *mec*C [120]. The *mec*C gene resides on a novel SCC*mec* element designated SCC*mec* XI [121]. Methicillin-resistant *S. aureus* strains carrying the *mec*C gene have been shown to cause a range of infections in humans and appear to be predominantly community associated [118, 119]. The prevalence of *mec*C in CNS has not been extensively explored as yet [60], but an allotype of the *mec*C gene has been detected in a *S. xylosus* strain [118].

6.3.1. Healthcare-associated MRSA

Traditionally, MRSA has been considered a hospital- or healthcare-associated pathogen (HA-MRSA) primarily infecting people who are immunocompromised or who have had surgery or medical device implants [122, 123]. Healthcare-associated MRSA strains usually carry SCC*mec* types I, II and III and are multidrug resistant [14]. Worldwide, the majority of HA-MRSA strains belong to CC5, CC8, CC22, CC30 and CC45 [14, 122].

6.3.2. Community-associated MRSA

Since the mid-1990s, MRSA strains were increasingly reported in healthy people without any healthcare-associated risk factors [31, 122]. These cases were termed community-associated MRSA (CA-MRSA), and genetic analyses revealed that these *S. aureus* isolates were genetically distinct from the typical HA-MRSA strains [31]. Community-associated MRSA strains are primarily associated with SCC*mec* types IV and V, which typically lack non-β-lactam resistance genes [124]. Most CA-MRSA strains belong to sequence type (ST)1, ST8, ST30, ST59, ST80 and ST93 [14, 122] with ST8 ('USA300') being the most common clonal lineage in the USA and ST80 the most common in Europe [125, 126]. Carriage of the gene encoding the Panton–Valentine leukocidin appears to be epidemiologically associated with certain CA-MRSA strains [14, 123].

6.3.3. Livestock-associated MRSA

The emergence of a third group of MRSA strains was witnessed in the last decade, which was described following investigations that began on a pig farm in the Netherlands [54, 127]. Pig farmers and other close human contacts were found to be at a higher risk of carrying MRSA than members of the population who did not frequent pig farms [128]. This group of MRSA strains, initially referred to as 'non-typeable MRSA' or 'pig MRSA', was found to belong to a single clonal complex, CC398, with the majority of strains belonging to sequence type (ST)398 [31]. Methicillin-resistant *S. aureus* ST398 has subsequently been isolated from other animal species, including dogs, horses, veal calves and poultry [125, 129–131], and it has therefore been designated livestock-associated MRSA (LA-MRSA) [125]. It has been shown that persons in direct (occupational) contact with LA-MRSA-positive animals, such as farmers, laborers, veterinarians and abattoir staff, have an increased risk of becoming MRSA carriers [31]. Methicillin-resistant *S. aureus* ST398 strains can cause infections in both animals [31] and humans [117]. Furthermore, certain strains belonging to an independent clade within CC398 have been associated with direct human-to-human transmission without prior exposure to livestock [132].

Livestock-associated MRSA ST398 carries SCC*mec* element IV or V [133]. These strains are generally resistant to tetracycline while resistance to aminoglycosides, lincosamides, macrolides and trimethoprim has also been documented [31]. Fluoroquinolone resistance has also been reported in isolates from Germany [14]. The LA-MRSA ST398 strains have been found to carry previously unidentified resistance genes, such as *dfr*K, a novel, plasmid-borne trimethoprim resistance gene [134]. This gene is located close to *tet*L, which would allow for the selection of either gene by the use of tetracycline or trimethoprim, both of which are used in veterinary medicine [135]. A novel ABC efflux pump encoding gene, *vga*C, which confers resistance to lincosamides and streptogramins, was also found on the same plasmid [134]. The multidrug resistance gene, *cfr*, was found in two porcine *S. aureus* isolates from Germany, one MRSA ST398 and one MSSA ST9 [136]. The *cfr* gene confers resistance to a number of antimicrobials including lincosamides, oxazolidinones, pheni-cols pleuromutilins and streptogramin A [133].

Molecular typing and whole genome sequencing have revealed that LA-MRSA CC398 strains originated from human-methicillin-sensitive *S. aureus* strains, which crossed the species barrier and in the process lost phage-carrying virulence genes that are usually found in human isolates [137]. The host switch from humans to livestock was further accompanied by the acquisition of methicillin and tetracycline resistance genes [137], suggesting that an antibiotic selective pressure exists in the livestock industry [138].

6.4. Mechanisms of vancomycin resistance in staphylococci

The molecular mechanisms underlying VISA and VRSA are different [139, 140]. Intermediate vancomycin resistance is associated with the presence of a thickened and/or poorly cross-linked peptidoglycan bacterial cell wall [140]. The altered cell wall structure traps the antimicrobial molecules reducing cellular penetration and preventing the antimicrobial from reaching its target site [140]. Heteroresistant VISA isolates (hVISA) have been described by

Hiramatsu and co-workers [141]. Heteroresistant strains are susceptible to vancomycin but contain a small subpopulation of cells, approximately one in every 10^6 cells, which exhibit resistance. It is proposed that hVISA may be a precursor to VISA and, as such, needs to be detected so that appropriate control measures can be implemented to limit the spread of the bacterium [142].

Vancomycin-resistant *S. aureus* strains do not arise from VISA but have acquired the complete genetic apparatus mediating resistance to glycopeptides from vancomycin-resistant entero-cocci [51, 93, 98]. The genes encoding vancomycin resistance, collectively referred to as the *van*A gene complex, reside on a transposon, Tn*1546* [139]. The transposon is carried by a conjugative plasmid and is transmissible to a number of Gram-positive bacterial genera including *Bacillus*, *Staphylococcus* and *Streptococcus* [139].

7. Alternate bacterial strategies to circumvent the action of antimicrobials

In addition to the challenges posed by antimicrobial resistance, the treatment of staphylococcal infections is further complicated by a number of strategies that staphylococci have developed, which enable the bacteria to evade the host immune response and the activity of antimicrobials [12, 143]. Two strategies, namely the formation of biofilms and the development of small-colony variants, will be discussed in further detail.

7.1. The formation of biofilms

Biofilms can be described as large, amorphous aggregates of bacterial cells encased in extra-cellular material comprising *inter alia*, bacterial by-products, polysaccharides and proteins [12]. Biofilms may form on abiotic surfaces, such as implanted medical devices as well as biotic surfaces, such as host tissue [12, 39]. The formation of biofilms can be visualized as being a four-step process: (i) the attachment of bacteria to the surface; (ii) proliferation of the bacterial cells; (iii) biofilm growth and maturation; and finally (iv) dissociation and dissemination of bacterial cells to new sites [12, 39].

The formation of biofilms affords bacterial cell protection from a multitude of chemical, cellular and physical antagonists [143]. The bacteria encased in biofilms are able to tolerate significantly higher concentrations of antimicrobials and disinfectants than free-floating bacterial cells [39, 143, 144]. Furthermore, the bacterial cells residing in biofilms are more resistant to phagocy-tosis and are protected from pH extremes and physical desiccation [143]. The protective effect of biofilms is in part attributable to the physiological changes that the bacterial cells undergo whilst growing *en masse*. Bacteria existing within biofilms grow more slowly than exponential-phase bacteria [143]. This is partly due to restricted diffusion of gases and nutrients within the biofilm environment, but this is also affected by alterations in bacterial gene expression [145]. Beenken and co-workers [145] revealed a change in the expression of 580 genes (more than 20% of the genome) when using microarrays to study differences between *S. aureus* cells growing in biofilm and planktonic cultures.

The close contact between bacterial cells residing in biofilm communities facilitates and promotes the exchange of MGEs [146]. The horizontal transfer of plasmids in biofilms is typically higher than observed between cells existing in a planktonic state and, in fact, studies have shown that biofilms promote plasmid stability and may enhance the host range of MGEs [146]. As previously discussed, the exchange of MGEs plays a significant role in the emergence of new, potentially more virulent, staphylococcal strains.

7.2. Intracellular persistence and the formation of small-colony variants

The ability of staphylococci to persist intracellularly in non-professional phagocyctic cells following ingestion affords protection to the bacteria from the host immune system as well as the action of antimicrobials [12]. The adaptation to an intracellular environment is accompanied by the formation of 'small-colony variants' (SCVs), which represent an alternate phenotypic and metabolic state of the normal, wild-type, staphylococcal phenotype [12, 147]. The SCV phenotype is characterized by a reduced growth rate as well as substantial changes in gene expression [12]. The altered phenotypic state also affects the susceptibility of the bacteria to antimicrobials [144]. In addition to phagocytes, internalization of *S. epidermidis* in human endothelial cells and bone cells has been demonstrated [12].

The formation of biofilms and small-colony variants is implicated in persistent and relapsing infections, and, as such, it poses a significant challenge for the treatment staphylococcal infections [12, 147].

8. Use of antimicrobials in animal health and food animal production operations and implications for human health

Antimicrobials are used in animal health and food production to treat and prevent disease and, more contentiously, for growth promotion in food production animals [148, 149]. The volume of antimicrobials used in animals is larger than the volumes used in human medicine even in countries where strict regulations regarding antimicrobials are enforced [148]. Exact data on antimicrobial consumption in animals are scarce and only available for a few countries [148]. Recent data from the USA suggest that almost 80% of antimicrobials produced are used in food-producing animal operations [150–152] and 70% hereof are used for non-therapeutic purposes [153, 154]. The largest users of antimicrobials are typically the poultry and swine producers due to the intensive nature of these production systems [155].

The use and administration of antimicrobials in companion animals (cats, dogs and horses) fall largely under the control of veterinary practitioners [148, 156]. Individual animals are examined and diagnosed, following which the appropriate therapeutic recourse is selected [148, 156]. In the event that antimicrobials are administered, this is done in accordance with the manufacturer's recommendations ensuring the prudent use of antimicrobials [148]. In contrast, the use of antimicrobials in food production animals (livestock and poultry) is often done with little or no veterinary consultation [148]. Many antimicrobials are accessible to

producers as 'over-the-counter' remedies from local retailers, thereby limiting the control over the use of these products [148, 157]. In food production animals, antimicrobials may be applied therapeutically to treat sick individuals, but it is more common for producers to apply antimicrobials to entire herds or flocks in order to treat sick animals and to curb the spread of infectious organisms to healthy animals [148, 156]. The administration of antimicrobials in this manner is termed metaphylaxis [148, 158].

In food production systems, antimicrobials are often intentionally administered to animals in sub-therapeutic doses to promote growth and enhance feed efficiency [148]. The benefits of using antimicrobials as 'growth promoters' were recognized as early as the 1940s [149, 158]. Researchers observed that poultry that were administered vitamin B12 in the form of crude *Streptococcus aureofaciens* fermentations showed improved growth compared to birds given purified vitamin B12 [159]. It was speculated that the crude fermentations contained an unidentified growth factor, which enhanced growth [158]. The growth factor in the fermentation product was subsequently identified as chlortetracycline [158]. Shortly after this observation, the US Food and Drug Administration (FDA) approved the inclusion of certain antimicrobials into animal feed to enhance animal growth and production as well as prevent disease [158]. Some of the antimicrobials which have been utilized as growth promoters in some countries include: avilamycin (everninomycin), avoparcin (glycopeptide), bacitracin (polypeptide), bambermycin (glycolipid), carbadox and olaquindox (quinoxalines), lincomycin (lincosamides), pencillin (β-lactams), streptomycin (aminoglycosides), tetracycline and chlortetracycline (tetracyclines), tylosin and spiramycin (macrolides) and virginiamycin (streptogramin) [156, 159, 160].

The use of antimicrobials in animals, particularly as growth promoters in food producing animals, has been subjected to intense scrutiny and is frequently criticized as a driving force behind the emergence, maintenance and horizontal transfer of antimicrobial-resistant determinants in bacteria [161, 162]. The principle concern is the potential zoonotic transmission of antimicrobial-resistant pathogenic and non-pathogenic bacteria to humans either through direct contact with animals or indirectly through contact with the animals' environment or through the food chain [161, 163]. Due to public concerns and increasing scientific evidence, stricter regulations regarding the use of growth promoters have been implemented [164]. The European Union began phasing out the use of antimicrobials for growth promotion in the late 1990s [163]. By the year 2000, Denmark had successfully implemented a complete ban of antimicrobial growth promoters in food animal production [157, 160]. Stakeholders in favor of restrictions have argued that in countries like Denmark, where bans have been introduced, there has been a concomitant decrease in antimicrobial resistance in animal and human bacterial isolates [164]. Opponents to the ban of growth promoters have, however, questioned the evidence provided by supporters of the ban and have argued that a decline in the use of growth promoters will negatively affect productivity and animal health, which will in turn lead to an increase in the therapeutic use of antimicrobials [149, 164]. A number of excellent reviews have examined the complexity and debate surrounding the use of growth promoters in livestock production, and the reader is referred to these texts for further information [148, 149, 157, 165–167].

9. The interface between human and animal populations

The dynamics of staphylococcal antimicrobial resistance and bacterial transmission at the human–animal interface will be considered separately for companion animals (cats, dogs and horses) and food production animals (livestock and poultry). Consideration will also be given to the intersection of humans and animal carcasses further along the food chain in the abattoir.

9.1. Companion animals

It is common in developed countries for humans to own companion animals [126]. Due to the close contact between humans and their pets, the opportunity for the transmission of bacteria between hosts is high [126]. Numerous reports have documented the transmission of MRSA strains between humans and dogs [168–174], humans and cats [175, 176] and humans and horses [177, 178]. Bacterial transmission of MRSA leads to both hosts becoming colonized, which places the hosts at a higher risk of being infected by the colonizing strain when the opportunity presents [78]. Furthermore, the colonized hosts serve as reservoirs of MRSA for other members of the household [179, 180].

Molecular genotyping of MRSA isolates recovered from companion animals has revealed that the *S. aureus* strains recovered from colonized and infected animals usually belong to clonal complexes implicated in human infections [126, 171, 173, 174, 177, 181, 182]. An investigation conducted in the United Kingdom examined the occurrence and the genetic relatedness of MRSA recovered from veterinary personnel and hospitalized animals in a small animal hospital [171]. Eighty-two percent (23/28) of the MRSA isolates recovered from the nasal mucosa of staff, hospitalized dogs and the environment were genetically related to EMRSA-15 (ST22), the predominant MRSA clone responsible for nosocomial infections in the United Kingdom [171]. In the USA, the most common MRSA clone recovered from companion animals is the ST5 clone, which is also the most common HA-MRSA clone in humans [171]. These data have suggested that the transmission of MRSA usually occurs from the human host to their respective pet [78, 179].

In general, prevalence studies seem to suggest that MRSA colonization amongst healthy pets is low [126, 176]. Higher MRSA colonization rates have been documented amongst companion animals in settings like animal shelters and veterinary hospitals [126, 171, 183]. Presumably, due to the relatively low MRSA colonization of companion animals there is currently no significant evidence indicating that pet owners are at an increased risk of MRSA colonization or infection compared with humans who do not own pets [107]. However, it is suggested that the lack of evidence may be partly attributed to the paucity of studies examining this particular aspect of animal ownership [107].

Since 2006, there has been a significant increase in the number of documented cases involving the isolation of methicillin-resistant *S. pseudintermedius* (MRSP) strains from surgical wound infections of dogs and cats [79]. A few studies have reported the occurrence of indistinguishable strains of MRSP from humans and their canine companions [176] and amongst animals and workers in veterinary clinics [184]. In Japan, a study investigating the prevalence of MRSP

in a veterinary teaching hospital, cultured MRSP from 17 dogs and a staff member [25]. The isolate recovered from the employee had an antimicrobial susceptibility pattern and a PFGE profile similar to isolates recovered from dogs handled at the facility, indicating zoonotic transmission [25]. A study investigating the prevalence of MRSP in staff working at a veterinary dermatology practice reported that 5.3% (9/171) of the staff tested positive [185]. In general, owners of infected pets and veterinarians handling infected animals seem to have a higher risk of being MRSP positive [79]. In all documented cases, MRSP-positive individuals have been asymptomatic [79].

9.2. Food production animals

Livestock and poultry production has, over the past few decades, intensified in order to keep abreast with the food demands posed by a burgeoning human population [138, 148]. Larger numbers of animals are maintained under confined conditions in order to maximize productivity and improve profit margins. Accompanying these changes in farming practices has been an increase in the use of antimicrobials as well as increase in the proximity in which animals and humans co-exist [137, 138]. The close proximity of animal and human hosts has in turn increased opportunities for the transmission and exchange of microbial flora [56, 138]. It is well established that individuals such as farmers, veterinarians, farm laborers and abattoir workers working in close contact with animals have a greater risk of being colonized or even infected with zoonotic bacteria carried by animals than individuals that do not interact with animals [31]. The relatively recent description of zoonotic LA-MRSA ST398 and the novel methicillin resistance gene, *mec*C, has once again highlighted the implications associated with the horizontal transmission of pathogenic bacteria between animal and human hosts and the role of animals in the epidemiology and the evolution of human disease [118, 186].

Since the description of LA-MRSA, a plethora of studies have been conducted to estimate the prevalence of MRSA in different food animals, and a number of reviews have been published [14, 31, 107, 125]. Livestock-associated MRSA has been extensively described in pig production systems with many investigations documenting the transmission of CC398 between animals and close human contacts [187–193]. A study conducted in Germany found 86% (97/113) of people who worked with pigs to be asymptomatic carriers of CC398 MRSA [190]. Interestingly, in the same study, sampling of the family members of CC398 MRSA carriers showed that 4.3% (5/116) of these individuals, who had no direct exposure to pigs, were colonized by the same MRSA strain [190]. Nasal colonization was also found in 45% (22/49) of veterinarians frequenting pig farms in the study area and in 9% (4/44) of their family members who had not been exposed to pigs [190]. A pilot study conducted in two large pig production systems in the USA revealed an overall MRSA prevalence of 49% (147/299) in the animals sampled and 45% (9/20) of the farm workers [194]. All MRSA isolates belonged to ST398 [194]. In Belgium, 37.8% (48/129) of close human contacts sampled from 50 pig farms were found to be colonized by MRSA ST398 [191]. An identical MRSA strain was recovered from the skin lesions of one worker who was sampled at the time of the study [191]. In some geographical settings, other MRSA clones have been found to colonize pigs. In China, MRSA strains belonging to ST9 were commonly isolated from pigs and close human contacts [195, 196]. In Italy, pigs sampled at

abattoirs were found to be colonized by MRSA ST9, ST(CC)97 and ST398 [197]. Further, the presence of human-associated CA-MRSA t127, ST1 and SCC*mec* type V was detected [197]. Despite the high rate of LA-MRSA colonization in pigs, this *S. aureus* clone has only been implicated in sporadic clinical infections in pigs [107]. Livestock-associated MRSA has been isolated from skin infections such as exudative epidermidis and infections of the urogenital tract and the uterus and mammary gland of pigs [14].

In the Netherlands, MRSA ST398 colonization has been documented in veal calves and close human contacts [131]. From the 102 farms sampled in one study, MRSA was isolated from animals on 88% (90/102) of the farms investigated [131]. Overall, 28% (602/2151) of the animals and 33% (32/97) of the farmers sampled tested positive for MRSA [131]. The MRSA strains recovered from the human and animal samples included ST398 as well as ST5, ST15, ST45 and CC34 from the human specimens and ST97, ST239, ST1159 and CC425 from the calves [131]. The data clearly demonstrated that MRSA colonization of human contacts was strongly associated with the intensity of animal contact and with the number of MRSA-positive animals on the farm [131]. Furthermore, a direct correlation was observed between MRSA prevalence and farm hygiene practices [131].

In dairy cattle operations, LA-MRSA ST398 has been isolated from clinical and subclinical milk samples. One of the first reports, emanating from Belgium, recovered LA-MRSA from 9.3% (11/118) of the dairy farms surveyed [198]. The prevalence on positive farms varied between 3.9% and 7.4% [198]. Methicillin-resistant *S. aureus* ST398 has also been reported from dairy herds in Germany [199] and Switzerland [61, 200]. Juhász-Kaszanyitzky and co-workers [201] published the first report documenting the transmission of MRSA between dairy cows with mastitis and a close contact worker on the farm. In this study, identical MRSA strains, belonging to MLST ST1, were recovered from both animal and human hosts. The direction of transmission, from bovine to human or human to bovine could, however, not be established [201].

One of the first reports of LA-MRSA in poultry emerged following a study conducted in Belgium [130]. The researchers reported that 12% (10/81) of the *S. aureus* isolates recovered from the nasal and cloacal swabs of healthy broiler chickens belonged to *spa* types associated with CC398 [130]. A further study in Belgium identified a new *spa* type, t1456, within CC398 following a random sampling of broiler farms [202]. Despite sporadic reports on the isolation of CC398 from poultry operations, the epidemiology of LA-MRSA in poultry is still unclear [14].

According to Schwabe [203], a zoonosis is described as a 'shared infection' of animals and man, without ascribing direction of transmission from one host to the other. Inasmuch as bacterial transfer and colonization, or infection, of humans are of significant concern to human medicine, the reverse scenario, which is often overlooked, warrants consideration. Humans may represent an important source of new bacterial strains, which can cause disease in livestock and, as such, pose a potential threat to food security [138]. Several molecular genotyping studies, which have traced the origins of epidemic *S. aureus* clones in human and animal hosts, have reported that the majority of host-switch events have involved the movement and adaptation of bacteria from human to animal hosts [62]. Both

LA-MRSA ST398 and the major pathogenic *S. aureus* ST5 clone, responsible for lameness in poultry, have been shown to originate from humans but have now adapted and diversified to spread in animal hosts [58, 137].

Irrespective of the direction of bacterial transmission, it is of mutual benefit to both human and animal health that bacterial populations at the interface between different host species are monitored. Surveillance is therefore advocated in order to monitor changes in the epidemiology and virulence of bacterial strains and to enable appropriate pre-emptive measures to be taken [138].

9.3. Food animal products

The abattoir environment presents a dynamic interface between humans and animals largely due to the fact that abattoirs process large numbers of animals originating from different farms across a relatively broad geographic expanse. During slaughtering and subsequent processing, it is quite plausible for carcasses to become contaminated with staphylococcal strains origi-nating from animals, abattoir workers or the environment [14]. Numerous reports have documented the occurrence of CA-MRSA, HA-MRSA and LA-MRSA strains from different meat products [14, 204, 205, 206]. An extensive study conducted in the Netherlands demon-strated the presence of LA-MRSA on a variety of raw meat products collected from retail outlets [204]. In this study, 11.9% (264/2217) of the raw meat products analyzed were found to be positive for MRSA [204]. It was further shown that 85% (224/264) of the MRSA strains belonged to *spa* types associated with CC398 [204]. A survey conducted in the USA found 39.2% (47/120) of the retail meat samples analyzed to be positive for *S. aureus*. Five percent (6/120) of the *S. aureus* isolates were resistant to methicillin. Molecular typing identified the isolates as belonging to the ST5 and ST8 lineages [205]. A similar survey conducted in Canada found 7.7% (31/402) of the meat samples analyzed contained MRSA [206, 207]. The three major sequence types obtained were ST5 (29%), ST8 (39%) and ST398 (32%) [206, 207].

The principal concern arising from the presence of MRSA in food is the development of food poisoning following ingestion of preformed staphylococcal enterotoxins [207]. The best preventative measure is to ensure the correct handling and storage of food to reduce the risk of enterotoxins being produced [207]. Another concern regarding the presence of MRSA on meat is that people may become colonized or infected from handling or eating contaminated meat [205, 207]. Regarding the latter, there is, at present, no substantial data to support or refute this concern. More intensive surveillance is needed to elucidate the true role of food contam-ination in the development of human diseases [107].

10. Monitoring antimicrobial resistance in staphylococci at the human–animal interface

In addition to direct contact between animal and human hosts, the transmission of antimicro-bial-resistant bacteria and resistance genes may occur through a number of routes [76, 149].

Figure 3 presents some of the potential routes of bacterial transmission taking into consideration the role of the environment as well as aspects related to the movement of animals, food products and human contacts. The globalized trade of live animals and/or meat products is one of the features of modern food production systems, which has the potential to elaborate the impact of antimicrobial-resistant bacteria of animal origin [138].
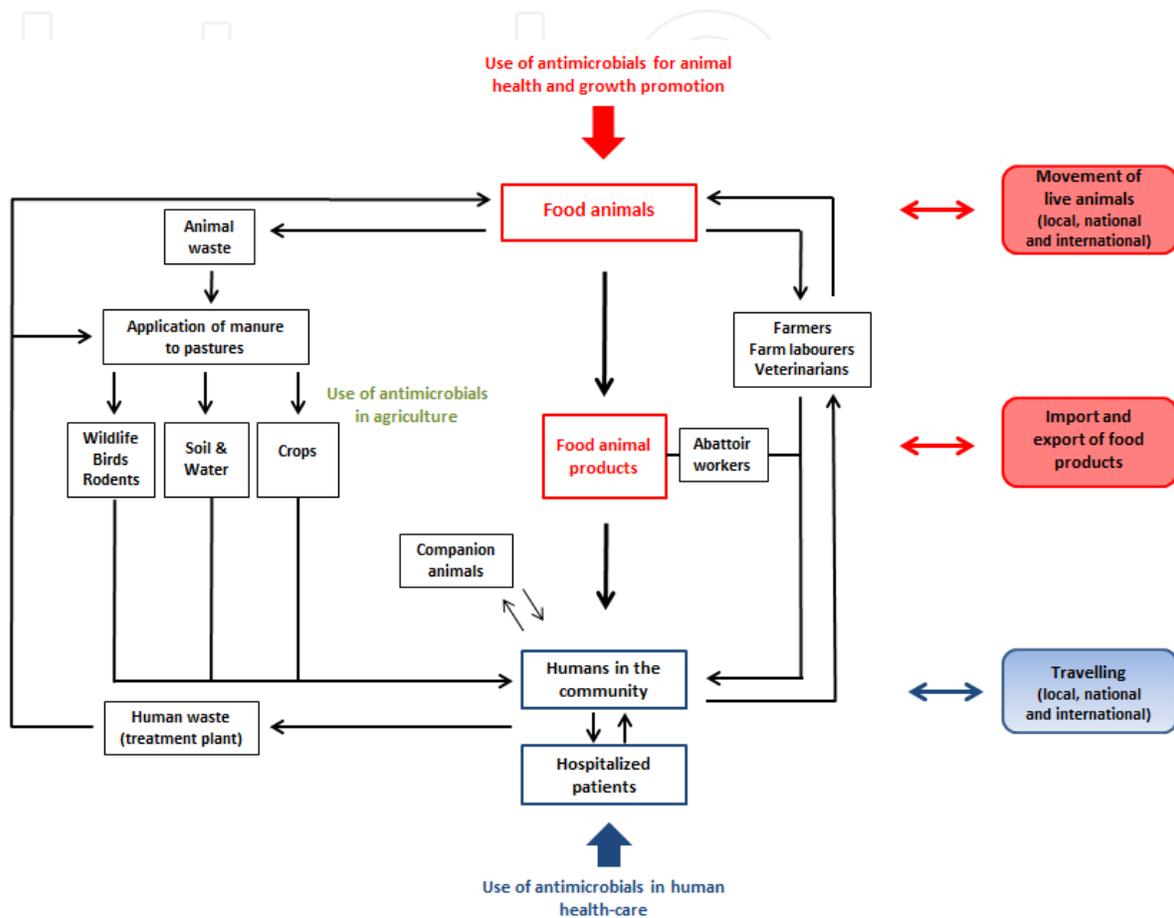
Figure 3. Potential routes of dissemination of antimicrobial-resistant bacteria and antimicrobial resistance genes [76, 149]. ©2005 American Society for Microbiology. Adapted with permission. No further reproduction or distribution is permitted without the prior written permission of American Society for Microbiology

In order to be able to accurately assess the impact of antimicrobial use in animal health and food production operations on human medicine, integrated surveillance programmes are needed [148]. The formulation and implementation of surveillance programmes require a concerted effort from role-players in multiple disciplines. Funding, infrastructure, political 'buy-in' and the support of several role-players are key to the success of these programs [148]. Currently, only a few countries have been able to implement successful monitoring programmes [148]. One of the longest running and most successful programmes is the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP), which has been systematically collecting and analyzing data since 1995 [163]. The programme utilizes a 'one-health' approach and entails the monitoring of the entire food chain from 'farm to fork to sickbed' [208]. The objectives of DANMAP are summarized as follows:

- to monitor the use of antimicrobial agents in food animals and humans;

- to monitor the occurrence of antimicrobial resistance in bacteria isolated from food animals, food of animal origin and humans;

- to study associations between antimicrobial consumption and antimicrobial resistance; and

- to identify routes of transmission of resistant bacteria and/or resistance determinants and areas for further research [163, 208].

Results from DANMAP are reported annually and are accessible online [163]. The data accumulated from long-term surveillance programmes should enable resistance trends to be monitored over periods of time as well as identify emerging problems so that adequate intervention strategies can be implemented [148, 208].

In accordance with Office International des Epizooties (OIE) guidelines, surveillance programmes should investigate antimicrobial resistance in the following groups of bacteria:

1. Human and animal pathogens that cause infections. These bacteria are thought to reflect resistance caused by the use of antimicrobials in the respective reservoirs.

2. Zoonotic bacteria that can develop resistance in the animal reservoir and which can be transmitted to humans via direct contact or consumption of contaminated food. These bacteria may subsequently compromise treatment when causing infection in humans.

3. Sentinel or indicator bacteria, such as enterococci and *Escherichia coli*, are selected for monitoring purposes due to their ubiquity in animals, foods and humans. Furthermore, these bacteria readily develop or transfer antimicrobial resistance in response to selective pressure in both human and animals and are considered reservoirs of resistance determinants [148, 208, 209].

The staphylococci of animal origin which are commonly monitored as part of surveillance programmes include *S. aureus* and coagulase-negative staphylococci from bovine mastitis cases as well as *S. hyicus* isolates from cases of exudative epidermitis in pigs [148, 163]. From human health laboratories, *S. aureus* isolates derived from blood, urine and CSF samples are used for surveillance purposes [208, 209]. Some surveillance programmes, such as the British Society for Antimicrobial Chemotherapy Resistance Surveillance project, extend monitoring to include CNS species [209].

11. Evaluating the antimicrobial susceptibility of staphylococcal isolates

In a clinical context, evaluating the antimicrobial susceptibility of bacterial isolates is an important aid for practitioners needing to make decisions regarding the appropriate therapeutic treatment of infected patients [88]. Antimicrobial susceptibility testing of bacterial isolates also provides essential data for surveillance programs as previously discussed. Several methodologies exist for evaluating the *in vitro* susceptibility of bacterial isolates to different classes of antimicrobials. The two principal methods used are agar disk diffusion and the broth

micro-dilution minimum inhibitory concentration (MIC) method [148, 210]. The agar disk diffusion method provides qualitative results that categorize isolates as susceptible, inter-mediate or resistant to the antimicrobial(s) under evaluation [135, 210]. The method is relatively cost-effective and flexible with respect to the panel of antimicrobials that can be selected for testing [135]. The MIC method may be performed in a variety of formats ranging from in-house prepared plates or broths to commercially available micro-dilution plates or gradient strips [210]. The MIC method provides a quantitative result expressed in micrograms per milliliter as well as a categorization of the bacterium as susceptible or resistant [135, 210]. Since the method is able to quantify antimicrobial susceptibility, the MIC is the preferred method for use in surveillance or epidemiological programs [148].

Irrespective of the test methodology selected, it is imperative that all antimicrobial suscepti-bility tests are conducted in accordance with the international standard being followed, namely the Clinical Laboratory Standards Institute (CLSI), the European Committee on Antimicrobial Susceptibility Testing (EUCAST) or other recognized national standards [210]. Furthermore, it should be ensured that all of the appropriate quality control measures advocated by the standard are implemented and rigorously followed [210].

In vitro methods for analyzing the antimicrobial susceptibility do not take into consideration the protective effect afforded by biofilm growth, which commonly occurs during the course of staphylococcal infections [143]. Due to the protective environment afforded by biofilm growth and the accompanying changes in bacterial physiology, bacterial cells growing in biofilms are typically able to tolerate antimicrobial concentrations 10-fold to 1000-fold higher than planktonic bacterial populations [143]. A few methods have evolved to investigate the antimicrobial susceptibility of isolates growing in biofilms, with the commercially available method from Innovotech Inc. (Edmonton, Canada) gaining wide acceptance. The Innovotech MBEC™ P&G system is a uniquely designed microtiter plate with 96 identical pegs protruding from the plastic lid (Innovotech Inc., 2012). The system facilitates the generation of 96 identical biofilms on the pegs which can be subjected to varying concentrations of antimicrobial or disinfectant to calculate the minimum biofilm eradication concentration (MBEC) and MIC values for each test isolate (Innovotech Inc., 2012). The Innovotech MBEC™ P&G system (formerly called the Calgary biofilm device) has been used in a number of applications to examine the effect of different chemicals on staphylococcal biofilms [211–215].

11.1. Detection of antimicrobial resistance genes in staphylococcal isolates

A complementary approach to phenotypically evaluating the susceptibility of staphylococcal isolates is to screen test isolates for specific antimicrobial resistance genes using molecular assays, such as PCR and real-time PCR [24, 216]. This approach is still only infrequently used in routine clinical diagnostic work, but from a research perspective, molecular screening has provided a wealth of information with respect to the distribution and spread of resistance genes amongst bacteria [88].

An obstacle to using DNA-based assays for resistance testing is the formidable complexity of resistance mechanisms that exist [217]. It is common for resistance to an antimicrobial to involve multiple genes and, in some cases, not all of the mechanisms involved have been

identified [217]. The use of microarrays is one manner in which this limitation has been overcome [216]. Microarray analysis enables bacterial isolates to be simultaneously screened for a large number of gene targets [216]. A DNA microarray consists of an orderly arrangement of DNA probes which have been spotted onto a solid support, such as a silicon chip, glass slide or nylon membrane [216]. Bacterial DNA or cDNA is labelled with a fluorescent dye and allowed to hybridize to the microarray [216]. One of the microarrays currently available commercially, the StaphyType Kit (Alere Technologies, Jena, Germany) permits the simultaneous screening of 334 *S. aureus* gene targets. In addition to screening for a multitude of antimicrobial resistance genes, the kit screens bacterial isolates for species-specific gene markers, toxin-encoding genes and genes encoding specific tissue-binding proteins [218].

12. Epidemiological molecular typing systems for staphylococci

Bacterial typing is important for both clinical and epidemiological investigations to determine the source(s) of infection, routes of transmission in disease outbreaks or the analysis of the genetic relatedness or specific characteristics of bacterial strains [219]. A number of different typing techniques have been developed, each with specific advantages and drawbacks. It is therefore imperative that the most appropriate method, or combination of methods, be selected depending on the purpose of the investigation on hand [219].

Pulsed-field gel electrophoresis is a highly discriminatory technique and is considered to be the 'gold standard' for typing *S. aureus* isolates of both human and animal origin [14]. Pulsed-field gel electrophoresis is also the recommended strain typing technique for *S. epidermidis* and other CNS [220]. Pulsed-field gel electrophoresis detects rapidly accumulating genetic variation and is therefore useful for distinguishing strains for the investigation of an outbreak or for examining the phylogeny of a small bacterial population [60]. The PFGE technique is based on the digestion of bacterial DNA with restriction enzymes that cleave specific recognition sites along the chromosome [221]. The restriction enzyme digestion generates a number of DNA fragments, which are resolved by electrophoresis in an electric field, which is pulsed at different angles across the gel. The resulting banding patterns are analyzed using specific software and interpretations made according to the criteria of Tenover and co-workers [222]. The principal drawbacks of this technique are the fact that this method is technically demanding and time consuming and requires several days before results are available [223]. Furthermore, specialized equipment is required to perform the gel electrophoresis [122, 223]. Interlaboratory reproducibility of results has also been problematic, making the comparison of data generated by different laboratories quite difficult [122].

Multilocus sequence typing (MLST) is a sequence-based genotyping method, which is performed by sequence analysis of approximately 450-bp internal fragments of seven housekeeping genes [224]. The DNA sequences for each locus are assigned distinct allele identification numbers, and the combination of the numbers defined for all loci is used to generate the sequence type (ST) [33, 122]. Isolates that have identical sequences at all seven loci are considered a clone, whereas sequence types that differ by single nucleotide polymorphisms

at fewer than three loci are considered closely related and are grouped in clonal complexes (CC) [14, 33]. In contrast to PFGE, MLST indexes genetic variations that accumulate slowly over time [125]. Multilocus sequence typing is, therefore, better suited to measure evolutionary changes over a relatively long time span and is the best method for studying the global epidemiology and frequency of specific bacterial lineages [60, 125]. The method is highly discriminatory and has the distinct advantage of enabling results from other laboratories and studies to be compared using the Internet [122, 224]. At present, the greatest limitation to using MLST is the high cost associated with the sequencing of multiple gene loci [125].

Typing of the *spa* gene is also widely used for the epidemiological study of *S. aureus* isolates [1]. The *spa* gene encodes protein A, an important virulence factor of *S. aureus* [225]. The typing method entails the DNA amplification and sequencing of a polymorphic 24-bp variable-number tandem repeat (VNTR) within the coding region of the *spa* gene followed by the assessment of the data using a central online server. Typing of the *spa* gene has been found to be a suitable typing method for conducting both local and global epidemiological studies [226]. This method has been found to have a greater discriminatory power than MLST but it is less discriminatory than PFGE [122, 227, 228]. Since *spa* typing involves the sequencing of only a single locus compared with MLST, it is cheaper, less laborious and less time consuming to perform [125, 228]. A potential problem, however, is that unrelated lineages can sometimes contain similar *spa* types [125].

Typing of the SCC*mec* element is one of the most important epidemiological tools for studying methicillin-resistant staphylococci [228]. A number of multiplex PCR and real-time PCR assays have been developed to classify the different SCC*mec* types and subtypes [229–234]. An overview of the scope and drawback of various SCC*mec* typing methods, which have been developed, can be found elsewhere [91]. At present, there is no universally accepted assay which can identify and differentiate all of the *mec* types and subtypes [122]. Furthermore, since the described PCR assays target different regions of the SCC*mec* element, it is not uncommon for discrepant results to be obtained when different methods are used to test the same isolates [228].

13. Concluding remarks

Staphylococci are ubiquitous in the environment and occur commensally on the skin and mucous membranes of humans and animal hosts. The genetic flexibility of bacteria in this genus, particular *S. aureus*, is the primary evolutionary driving force behind the emergence of new strains exhibiting enhanced virulence and antimicrobial resistance. The use of antimicrobials in animal health and food animal production operations has been implicated as a driving force behind the development of antimicrobial-resistant bacteria, which can transfer to humans through direct contact or indirectly through the food chain or environment. However, much of the evidence put forward to corroborate the argument against the use of antimicrobials in food animal production operations is tenuous and, if anything, it supports the need for further surveillance data. The implementation and maintenance of national and international inte-

grated antimicrobial resistance surveillance systems are required. Through active monitoring, potential problems can be identified and appropriate guidelines and policies put in place to ensure the longevity of clinically important antimicrobials.

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