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Antibiotic Resistance in *Staphylococcus* Species of Animal Origin

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1. Introduction

Staphylococcus is a genus of worldwide distributed bacteria correlated to several infectious of different sites in human and animals. Its importance is not only because of its distribution and pathogenicity but especially due to its ability to overcome antimicrobial effects. The goal of this chapter is to report data obtained from a decade of research in animal science field concerning to staphylococci antimicrobial resistance.

2. Characteristics and distribution of Staphylococci species in animals: An overview

The genus *Staphylococcus* is in the bacterial family Staphylococcaceae (Ludwig, 2009). Staphylococci are Gram-positive spherical bacteria that occur in clusters resembling grapes due to its perpendicular division planes where cells remains attached to one another following each successive division.

The genotypic standards for assigning an organism to the genus *Staphylococcus* include determination of guanine plus cytosine (G+C) content of 30-39mol% and phylogenetic trees constructed by comparison of 16S rRNA or 23R rRNA sequences (Takahashi et al., 1999). The phenotypic criteria is based on the ultrastructure and chemical composition of the cell wall, typical form Gram positive bacteria and catalase reaction positive for all species, except for *S. aureus* subsp *anaerobius* and *S. saccharolyticus*, which are strictly anaerobic. This genus comprises more than 50 species separated into two distinct groups based on their ability to produce coagulase. This topic approaches *Staphylococcus* spp distribution considering that different animal species have specific staphylococcal microbiota. Otherwise some clonal strains can colonize different animal species as is the case of methicillin-resistant *Staphylococcus aureus* (MRSA). Molecular techniques have been used to relate clonal groups of MRSA isolated from different animal species in order to understand its model of dissemination.

2.1 Coagulase-positive Staphylococci

This group includes the major pathogenic *Staphylococcus* species. *Staphylococcus aureus* is considered the most pathogenic one, especially due to its ability to produce a large range of virulence factors that enables it to colonize different tissues of a large range of animal species. The coagulase protein has the ability to turn fibrinogen into fibrin threads by a mechanism different from natural clotting (Palma et al., 1999). This protein is codified by the gene *coa* which possess a conserved and a repeated polymorphic region that can be used to measure relatedness among *Staphylococcus* coagulase positive isolates (Reinoso et al., 2006). The variable region of *coa* is comprised of 81-bp tandem short sequence repeats (SSRs) that are variable in both number and sequence, as determined by restriction fragment length polymorphism analysis of PCR products (Goh et al., 1992).

Staphylococcal protein A is a membrane-bound exoprotein characterized and well known for its ability to bind to the Fc region of immunoglobulins of most mammalian species. This protein is encoded by the *spaA* gene with a polymorphic (X) and a conserved region. The polymorphic X region consists of a variable number of repeated 24 pairs of bases located in the coding region for the cellular wall C-terminal extremity (Koreen et al., 2004). The diversity of the *spaA* short sequence repeat region seems to arise from deletion and duplication of the repetitive units and also by point mutation and this variation can be used in epidemiological studies. Frenay et al. (1994) reported epidemic MRSA strains with more than seven repeats and Montesinos et al. (2002) described isolates with 11 repeats as the most common type involved in an epidemic human outbreak caused by MRSA. The *coa* typing can be used to enhance the value of *spa* typing by providing more supported inferences on strain lineage and clonality among isolates with similar or identical *spa* repeat organization (Tenover et al., 1994). The use of more than one genetic marker for relating strains is desirable and likely to become increasingly important because recombination will eventually diversify resistant staphylococcal species to the extent that clonal types within a given region can no longer be distinguished by a single locus.

Staphylococcus aureus also produces others exoproteins that contribute to its ability to colonize host tissues such as slime and hemolysins. The adherence and fixation of *S. aureus* on biological surfaces represent the fundamental step in the development of infections. The production of slime mediates adhesion to implanted surfaces acting as a cementing matrix making bacteria less accessible to the host's defense system (Coelho et al, 2009). Slime production is controlled by the *ica* operon (*icaADBC*) and the co-expression of the *icaA* and *icaD* genes leads to a significant increase in such production (Arciola et al., 2001).

The alpha and beta hemolysins are important factors in the pathogenesis of Staphylococcal infections. The beta-toxin is an Mg^{2+}-dependent sphingomyelinase C which degrades sphingomyelin in the outer phospholipid layer of the membrane (Linehan et al., 2003).

The *agr* (*accessory gene regulator*) operon (*agrA, agrB, agrC agrD* and *hld)* is recognized as a quorum-sensing gene cluster that up-regulates production of secreted virulence factors such as alpha and beta-hemolysins, proteases, DNAses and sphingomyelinase. This same cluster also down-regulates the production of cell-associated virulence factors in a cell density-dependent manner in *S. aureus* (Lyon et al., 2000). The *agr* locus comprises two divergent transcriptional units under the control of the promoters P2 (RNA II) and P3 (RNA III). The

P3 transcript, an RNA III molecule, mediates the up-regulation of secreted virulence factors as well as the down-regulation of surface proteins (Novick, 2000).

Coelho et al. (2011) evaluated the presence of some *Staphylococcus aureus* virulence genes, including *coa*, *spaA*, *hla* e *hlb* in order to understand the distribution of *S. aureus* strains in dairy farms at Rio de Janeiro, Brazil, and contribute to the establishment of preventive strategies to reduce the spread of infection.

In veterinary medicine, others coagulase-positive staphylococci are reported as important pathogens, such as *Staphylococcus intermedius*, whose reclassification was proposed by Devriese et al. (2005), creating the *S. intermedius* group (SIG) including *S. intermedius,* a new specie *S. pseudintermedius* and *S. delphini*. Like *S. aureus*, the *S. intermedius* strains isolated from animals have been reported to produce an array of virulence factors, including leukotoxin, enterotoxin, and hemolysins, together with elements essential for biofilm formation (Futagawa-Saito et al., 2006). Besides SIG, others significative coagulase-positive in animals are *S. schleiferi* subsp. *coagulans, S. aureus* subsp. *anaerobius*.

2.2 Coagulase-negative Staphylococci

This group comprises the majority of *Staphylococcus* species. Coagulase-negative staphylococci (CNS), which were traditionally considered to be minor infectious pathogens, have become more common (Huxley et al., 2002). Several CNSs have been isolated from animal clinical specimens such as *Staphylococcus epidermidis*, *Staphylococcus simulans*, *Staphylococcus xylosus*, *Staphylococcus chromogenes*, *Staphylococcus warneri*, *Staphylococcus haemolyticus*, *Staphylococcus sciuri*, *Staphylococcus saprophyticus*, *Staphylococcus hominis, Staphylococcus caprae, Staphylococcus cohnii* subsp. *cohnii*, *Staphylococcus cohnii* subsp. *urealyticus*, *Staphylococcus capitis* subsp. *capitis* and *Staphylococcus capitis* subsp. *urealyticus* (Lilenbaum et al., 2000; Pereira et al., 2009; Pyöräla et al., 2009; Soares et al., 2008).

The conventional methods for CNS identification were primarily developed for human strains and their poor performance for identifying strains of animal origin seems to be related to a limited number of veterinary strains in databases (Bes et al., 2000). Additionally, the reference method developed by Bannerman (2003) is costly and too time consuming to be used in a clinical laboratory. Several molecular targets have been exploited for the molecular identification of *Staphylococcus* species, including the *groEL* gene (Goh et al., 1996). This gene, which encodes a 60-kDa polypeptide (known as GroEL, 60-kDa chaperonin, or HSP60 for heat shock protein 60) has the potential to serve as a general phylogenic marker because of its ubiquity and conservation in nature (Segal & Ron, 1996). Also it was proven to be an ideal universal DNA target for identification to the species level because it has well-conserved DNA sequences within a given species, but with sufficient sequence variations to allow for species-specific identification (Goh et al., 1996). Santos et al., (2008) had successfully used the *groEL* gene as a tool for the identification of the main *Staphylococcus* coagulase-negative species by PCR restriction fragment length polymorphism (RFLP). This group investigated 54 cows from 23 dairy herds located in the Brazilian States of Minas Gerais, Rio de Janeiro and São Paulo, between 1995 and 2003 and concluded that this gene constitutes a reliable and reproducible molecular method for identification of CNS species responsible for bovine mastitis.

2.3 Staphylococcal infections in animals

Reports of the importance of *Staphylococcus* species as pathogens in animal infections have been described and appear to be increasing. Among coagulase-positive species, *Staphylococcus aureus* is a cause of mastitis, dermatitis and suppurative conditions in several animal species. *S. aureus* causing mastitis is widely distributed in cattle, goats and sheep. The infection is often subclinical in cattle, leading to reduced milk production and milk quality, but acute catarrhal or even gangrenous inflammation may also occur.

For a long time, *S. intermedius* had been majorly considered a primary cause of pyoderma in dogs. It has also been reported to be involved in other diseases, such as pyometra, otitis externa and purulent infections of the joints, eyelids and conjunctiva (Werckenthin et al., 2001). Nowadays, a new classification was proposed by Devriese et al. (2005), creating the *S. intermedius* group (SIG) including *S. intermedius,* a new specie *S. pseudintermedius* and *S. delphini*. According to Sasaki et al. (2007), *S. pseudintermedius* is actually the major specie involved in this pathology. Futhermore, according to these authors, *S. intermedius* is restricted to feral pigeons and *S. delphini* which was usually described as the cause of suppurative skin lesions in dolphins is now considered to be involved in a larger spectrum of infectious animal diseases. *S. aureus* subsp. *anaerobius* has been implicated in lymphadenitis in sheep and *S. schleiferi* subsp. *coagulans* in external otitis in dogs.

Coagulase Negative Staphylococci (CNSs) have also been studied considering its potential pathogenicity for human and animals. Nowadays these bacteria are of great interest in veterinary medicine because they are currently considered emerging pathogens of bovine mastitis. Although CNS are not as pathogenic as the other principal mastitis pathogens and infection mostly remains subclinical, they can cause persistent infections, which result in increased milk somatic cell count (SCC) and decreased milk quality (Pyorala & Taponen, 2009). The prevalence of CNS mastitis is higher in primiparous cows than in older cows. Also this agent is implicated in the etiology of infectious diseases in household pets (Pereira et al., 2009). The most frequently isolated CNS species vary according to the geographical region under scrutiny and sample origin. In Brazil, Soares et al. (2008) detected prevalence of *S.xylosus* in mastitic milk samples, despite *S. chromogenes*, *S. simulans* and *S. epidermidis*, in general, appear to be the most frequently isolated CNS from mammary secretion samples worldwide (De Vliegher et al., 2003; Taponen et al., 2006).

Besides, *S. hyicus*, a variable coagulase producer, but mainly coagulase-negative, causes exudative epidermitis ("greasy pig disease") and an often acute generalized skin infection in piglets. Systemic forms of the disease which result in the death of the animals are also seen. Poor hygienic conditions as well as ec

toparasitic infestations favour the onset of the *S. hyicus* infection. Surviving piglets show retarded growth rates. In adult pigs, subacute skin infections, mastitis or metritis, but also septic arthritis may be caused by *S. hyicus* (Brückler et al., 1994). In goats and sheep however, enzootic acute gangrenous mastitis is commonly seen.

3. Antimicrobial resistance in Staphylococci of animal origin

Antibiotic resistance is the most puzzling question of public healthy in the earlier decade of this 21st century. Among bacteria this question seems to be more alarming due to its short

generation time and efficient gene recombination mechanisms. *Staphylococcus aureus* is the most representative example of how antibiotic resistance is a serious threatening worldwide. Nevertheless, all others coagulase-positive staphylococci are also able to develop resistance mechanisms to a large range of antimicrobials. Furthermore, the strains of several CNS species were also found to have high levels of resistance to various antibiotics.

This topic will discuss features of the resistance of staphylococci to antimicrobials, specially methicillin (oxacillin) and vancomicin, its mechanisms and epidemiology. Crucial questions about the use or abolishment of antibiotics used as "growth promoters" to food animal production will also be discussed.

3.1 Methicillin resistance

ß-lactamic antibiotics are the most frequently used in anti-staphylococcal infection therapy. Bacterial resistance mechanisms to this class of antibiotics include production of ß-lactamases and low-affinity penicillin-binding protein 2a (PBP2a) determined by the presence of the chromosomal genes *bla* and *mecA*, respectively. The latter, designated for methicillin resistance, precludes therapy with any of the currently available ß-lactam antibiotics, and may predict resistance to several classes of antibiotics (Moon et al., 2007). The isolation of *Staphylococcus aureus* methicillin- resistant (MRSA) from animals was first reported in 1972 following its detection in milk from mastitic cows (Devriese et al., 1972). Recent works reports a low incidence of MRSA mastitis and low prevalence of methicillin resistance among bovine *S. aureus* isolates (Juhász-Kaszanyitzky et al., 2007; Lee, 2003; Moon et al., 2007) so clinically it can be concluded that MRSA does not appear to be an important bovine mastitis pathogen. Nevertheless, the importance of epidemiological data concerns about MRSA in animals is reasonable and requires careful study in order to understand its emergence and dissemination. The long-term low prevalence of MRSA mastitis is quite surprising given the number of years since the first identification of MRSA in cattle and the close contact of humans with the udders of dairy cattle. Otherwise, several reports have been published showing MRSA infection in domestic animals, including dogs, cats, cattle, sheep, chickens, rabbits, and horses as an increasing trend (Goni et al., 2004; Hartmann et al., 1997; Lee, 2003; O'Mahony et al. 2005; Rich & Roberts 2004; Weese, 2005; Weese et al., 2006).

It is certain that animals are a source of human MRSA infection in some circumstances, but humans may also serve as sources of infection in animals. Exposure of household pets to MRSA was probably inevitable due to its increasingly prevalence in humans. Changes in the epidemiology of MRSA in unique specie may be reflected in changes in other species. The true scope of MRSA in animals and its impact on human health are still only superficially understood, but it is clear that MRSA is a potentially important veterinary and public health concern that requires a great deal more study to enhance understanding and effective response (Weese & Van Duijkeren, 2010). While most animals with MRSA are merely colonized, a wide range of clinical infections can occur. As would be expected with staphylococci, most MRSA infections in pets affect the skin and soft tissue. Wound infections, surgical site infections, pyoderma, otitis, and urinary tract infections are most common, but various other opportunistic infections have been reported (Baptiste et al., 2005; Griffeth & Morris, 2008). In a research developed by our team, 8% of MRSA was detected in a hundred of clinical specimens from different sites of household pets evaluated (Pereira et al., 2009).

Recently, a new MRSA was identified using high throughput DNA microarray screening. Complete genome sequencing revealed that this strain is distinctly different to previously described MRSA. It carries a new type of SCC*mec* encoding highly divergent genes that are very different to any described previously in MRSA or in any other organism. It was found to belong to the genetic lineage clonal complex 130 (CC130), which has previously only been associated with MSSA from cows and other animals, but not humans, strongly suggesting that the new MRSA originated in animals. (Gárcia-Alvarez et al., 2011)

Staphylococcal cassette chromosome *mec* (SCC*mec*) is a mobile genetic element composed by *mec* and *ccr* genes complex, which encodes methicillin resistance and the recombinases responsible for its mobility, respectively (Katayama et al., 2000). The expression of PBP-2a is controlled by regulator elements encoded by *mecR1* and *mecI* which are located adjacent to *mecA* on the chromosome. Deletion or mutation which occurred in *mec* regulator gene is considered to be associated with constitutive production of PBP-2a. Hence *Staphylococcus* spp possessing intact *mecRI* and *mecI* as well as *mecA* are phenotypically methicillin susceptible because of the repression of PBP-2a production by *mec* regulator elements. Such genomic changes in *mec* regulator genes are considered to alter or remove their repressor function on *mecA* gene transcription, which may lead to constitutive production of PBP-2a. The *mec* gene complex has been classified into four classes, and the *ccr* gene complex has been classified into three allotypes. Different combinations of *mec* and *ccr* gene complex types have so far defined six types of SCC*mec* elements (type I, II, III, IV, V) (Ito et al., 2004). It is important to analyze the genomic diversity found in *mec* regulator genes of staphylococci in order to understand the molecular basis for methicillin resistance.

The detection of methicillin resistance in routine clinical laboratories has been problematic ever since the emergence of MRSA during the 1960s and the difficulties are associated mainly with heterogeneous expression of resistance in most staphylococal strains currently prevalent (Witte et al., 2007). Misidentification of methicillin resistance can have serious adverse clinical consequences. False-susceptibility may result in treatment failure and in the spread of resistant *Staphylococcus* spp making it difficult to apply control measures and leading to the increasing of healthcare costs and may lead to overuse of glycopeptides (Velasco et al., 2005).

3.2 Vancomicin resistance

The glycopeptide vancomycin was first released in 1958. Vancomycin is an inhibitor of cell wall synthesis in *S. aureus* and other gram-positive organisms. While beta-lactam antibiotics inhibit cell wall synthesis by binding to the transpeptidase active site of penicillin binding proteins, vancomycin acts by a completely different mechanism so it has been the treatment of choice for serious infections caused by MRSA (Howden et al., 2010), but increase in vancomycin use has led to the emergence of two types of glycopeptide-resistant *S. aureus*. The first one, designated vancomycin intermediate resistant *S. aureus* (VISA) and the vancomycin-resistant S. aureus (VRSA).

The first report of clinical *S.aureus* isolate with reduced vancomycin susceptibility (VISA) was made by Hiramatsu et al. (1997) and generated great concern in the medical community. From there on, reports of strains of *S. aureus* (predominately MRSA) demonstrating the heterogeneous VISA (hVISA) or VISA phenotype have now been

reported for many countries including the United States, Japan, Australia, France, Scotland, Brazil, South Korea, Hong Kong, South Africa, Thailand, Israel, and others (Bierbaum et al. 1999; Chang et al., 2003; Denis et al. 2002; Ferraz et al., 2000 Gemmell, 2004; Howden et al., 2010; Kim et al., 2000; Perichon & Courvalin, 2006; Sng et al.,2005; Song et al., 2004; Tenover et al., 2004; Weigel et al., 2007)

Nowadays it is conceivable that VISA phenotype is related to the bacterial cell wall thickening, a passive resistance mechanism that reduces vancomycin access to its active site, which is localized in the cytoplasmic membrane in the division septum (Howden et al., 2010). It results in accumulation of acyl-D-alanyl-D-alanine (X-DAla-D-Ala) targets in the periphery that sequester glycopeptides (Cui et al., 2003).

Since 2002, nine methicillin-resistant *Staphylococcus aureus* (MRSA) strains that are also resistant to vancomycin (VRSA) have been reported in the United States. The fully vancomicin-resistant *Staphylococcus aureus* phenotype (VRSA) is due to acquisition from *Enterococcus* spp. of the *vanA* operon, carried by transposon Tn1546, resulting in high-level resistance (Arthur et al., 1993, Patel et al., 1997).

The emergence of enterococci vancomicin-resistant strains has been related to the use of avoparcin as growth promoter in swine culture (Aarestrup et al., 1996). Studies report the transfer of glycopeptide- and macrolide-resistance genes by transconjugation among enterococci and from *Enterococcus faecalis* to *S. aureus* (Młynarczyk et al., 2002). The vancomycin-resistance gene acquisition by *S. aureus* from *E. faecium* in the clinical environment has also been reported by Weigel et al. (2007). Recently, Tiwari & Sen (2006) have reported a VRSA which is *van* gene-negative.

In veterinary medicine, vancomycin-resistant enterococci were isolated from the feces of poultry and pig herds. It has significant impact in public healthy cause dissemination via contaminated animal food products possible (Aarestrup, 1995). These vancomycin-resistant *E. faecium* isolates from animals had decreased susceptibilities to avoparcin, a glycopeptide antibiotic widely used as a growth promoter in Western Europe and Australasia. Avoparcin is a fermentation product from a strain of *Streptomyces candidus* and is closely related to vancomycin (Aarestrup et al., 1996). Aarestrup et al (1996) showed that *E. faecium* isolates coresistant to vancomycin and avoparcin are commonly found in the feces of pigs and poultry in Denmark.

3.3 Others antimicrobials resistance

In Veterinary Medicine, clindamycin has been chosen as an antimicrobial alternative for the treatment of infections in dogs and cats caused by methicillin-resistant *Staphylococcus aureus* (MRSA) (Walther et al., 2008; Weese et al., 2006) and methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) (Schwarz et al., 2008; Wettstein et al., 2008). However, an inducible form of clindamycin-resistance may be present in some staphylococci. These staphylococcal strains appear susceptible on routine antimicrobial susceptibility testing, but resistance can be induced during treatment, possibly resulting in treatment failure (Swenson et al., 2007; Yilmaz et al., 2007).

Azithromycin have a remarkable application due to its superior pharmacokinetics properties and broad spectrum activity, including Gram-positive and negative bacteria species, intracellular pathogens and protozoan parasites. The principal characteristic that supported its

prescription and clinical significance is that it can be administrated by oral and parental routes, only by a single daily dose in short period of treatment facilitating veterinary therapy. Nevertheless, most of the knowledge that supports its use in veterinary therapy was based on studies that proved its therapeutic efficacy in human infections. Empirical antimicrobial chemotherapy without previous accomplishment of bacterial identification and antimicrobial susceptibility assays contributes to increase of antimicrobial resistance prevalence in pet animal reservoirs (Guardabassi et al., 2004; Morgan et al.; 2008).

Pereira et al. (2009) evaluated azithromycin resistance among 100 staphylococci isolates from pet animals infections. It was detected a percentual of resistant isolates of 54% to *Staphylococcus intermedius*, 67% of *S. aureus*, 38% of *S. hyicus*, 67% of coagulase-negative *Staphylococcus* spp through disc diffusion test. The variability of azithromycin susceptibility pattern is different to what is observed in humans infections whereas *Staphylococcus aureus* is the classical isolated pathogen. Broth dilution test detected azithromycin $MIC_{50/90}$ values of 16μg/mL, 64μg/mL in staphylococci isolates, respectivally, and 256μg/mL in Gram-negative rods (GNR). Agar dilution azithromycin $MIC_{50/90}$ values corresponded to 32μg/mL and 256μg/mL in staphylococci and >512μg/mL in GNR. Crescent azithromycin resistance rate has been previously reported. A study from 90's decade, detected azithromycin $MIC_{50/90}$ >128 μg/mL of MRSA isolated from human clinical samples (Neu, 1991). When comparing human MIC values reported in literature to data obtained from pet animal samples in the present study, azithromycin activity pattern may vary from different bacteria species and hosts, leading to therapeutic failures when classical human pathogens are adopted as reference to calculate dose and drug concentration. This justify microbiologic and pharmacokinetic assays to determine the specific azithromycin susceptibility breakpoints and therapeutic drug concentration to different bacteria species detected from animal disease.

It is important to point that MIC_{50} values varied among different staphylococci species, such as, *Staphylococcus intermedius* (32μg/mL), *S. hyicus* (32μg/mL), CNS (32μg/mL), from that value detected in *S. aureus* (16μg/mL) isolated from pet animal specimens.

Pereira et al. (2009) also evaluated the genetic markers of *Staphylococcus* spp azithromycin resistance by PCR technique and detected a 39% prevalence of *erm* genes, being *ermC* gene the most detected, showing a prevalence of 24% among all *Staphylococcus* isolates, followed by 12% *ermA* and 3% of *ermB*. No isolates were positive to *mefA* gene, what may support the theory about methylase ribossomal modification as the principal resistance mechanism associated to macrolide resistance among staphylococci. The expression of *erm* genes can be inducible or constitutive. When expression is constitutive, the staphylococci are resistant to all macrolide, lincosamide and streptogramin B (MLS_B) antimicrobials (Schmitz et al., 2000). In this study, it was detected MLS_B resistance in 14% constitutive azithromycin-resistant *Staphylococcus* spp. Inducible resistance phenotype, expressed by a "D zone" next to clindamycin disc was available in 5% (5/100) of *Staphylococcus* spp.

Most of the knowledge applied to define microbiologic use and dose of azithromycin was based on *Staphylococcus aureus* assays, because this specie acts as classical pathogen of human infections, but the frame of etiology and antimicrobial susceptibility pattern change when animal pathogens are considered.

Gentamicin is one of the most used antibiotics in dairy farm cattle. It is especially used as a prophylactic measure of mastitis control through intramammary injection. Its principal

resistance mechanism is mediated by the production of enzymes which transform aminoglycosides into inactive derivatives, such as acetyltranspherases, adenylyltranspherases and phosphotransferases. Modified aminoglycosides lose the ability to bind ribosomes and inhibit bacterial protein synthesis (Watanabe et al., 2009). Specific staphylococcal resistance to gentamicinis mediated by a bifuntional enzyme that acts as both acetyltranspherase and adenylyltranspherase. This enzyme is codified by the genes *aac* (*6'*)-*Ie* + *aph* (*2"*) which are transported in transposon Tn4001 located in plasmids of pSK1 family, conjugative plasmids pSK23 and in the chromosome (Udo & Dashti, 2000). Genetic elements Tn4001 are disseminated in *S. aureus* and CNSs. There is little information about its occurrence in staphylococci of animal origin (Lange et al., 2003).

3.4 Growth promoters

Antibiotic use in sub therapeutic levels as growth promoters is still common in Brazilian animal production. Defenders of this model believe that antimicrobial abolishment will result in higher morbidity, with a consequent raise of antimicrobial therapeutic use and consequently higher mortality. Also they think that it will directly implicate the efficiency of productivity as animals without growth promoters have a higher food consumer to achieve the same weight gain.

On the other hand, the European Economic Community established severe restrictions for products presenting antimicrobial residues defending the idea that this sub therapeutic use contributes to a positive selective pressure and to the spread of antimicrobial resistance genes between different pathogens. European Market defends that efficient animal handling is sufficient to control the infectious diseases and that avoid the probability of antimicrobial therapeutic failure. It is a highly controversial subject of extreme importance in a world concerned to the need of the improvement of food production. The experience of avoparcin use as growth promoter in some European countries and the consequent dissemination of a crossed-resistance to vancomicin in *Enterococcus faecium* and *E. faecalis* seems to be related to the adoption of these restrictive measures (Aarestrup et al., 1996)

Since 2003, Brazil instituted a work group in order to analyze and evaluate the use of substances such as carbadox, olaquindox, bacitracin zinc, spiramycin, virginiamycin and tylosin phosphate as animal feed additives products. In 2005, it also included the evaluation of avilamycin, flavomycin, enramycin, monensin and maduramycin.

The most efficient alternative to the antimicrobial indiscriminate use are probiotics. Probiotic acts in a significantly different mechanism from antimicrobials. They are thought to improve intestinal microbial balance through favoring the elimination of pathogenic bacteria and the proliferation of non pathogenic organisms. As a consequence it contributes to growth promotion without enhance antimicrobial resistance.

4. Advances in the field of nucleic acid-based techniques for the identification/typing and detection of antibiotic resistant *Staphylococcus* species

The importance of being able to identify staphylococci species routinely in clinical laboratories is increasing. However, the exact identification of CNS is not easy, because the biochemical traits of the species are very similar and many clinical isolates show

intermediate traits. Additionally, the use of commercial identification kits to identify staphylococci does not include all *Staphylococcus* species, and their reliability for certain species is not sufficient. Molecular methods of identification seem to be the key to fulfill these spaces, as gene specific markers are being recognized. In the same way, molecular techniques have been developed in order to improve the detection of antibiotic resistant bacterial strains. So, nucleic acid-based detection systems offer rapid and sensitive methods to detect the presence of resistance genes and play a critical role in the elucidation of resistance mechanisms. This topic will discuss the variety of nucleic-based techniques used for diagnostic applications and demonstrate that no universal technique exists which is optimal for detection of specific genes. The choice of a particular technique is also dependent on the information required or the targets under consideration, but some techniques are more favored than others.

The advantages of genotypic detection of antibiotic resistance and bacterial characterization include: (i) The search for a defined resistance determinant; (ii) Independence upon phenotypic categories such as susceptibility, intermediate susceptibility and resistance for which breakpoints may vary between countries; (iii) Detection of low-level resistance which is difficult to detect using phenotypic methods; (iv) Reduction of the detection time through its performance directly with clinical specimens. This is particularly important for difficult-to-culture organisms; (v) Reduction in detection time of slow growth of the organism; (vi) More precise and fast therapeutic predictions; (vii) Minor biohazard risk once it is not necessary to propagate by culture of a microorganism (Sundsfjord et al., 2004).

On the other hand, the genotypic approach contains certain limitations and pitfalls: (i) It is based on screening for resistance determinants whereas antimicrobial therapy is preferably based on the detection of susceptibility; (ii) You can only screen for what you already know so it does not take into account new resistance mechanisms; (iii) There are silent genes and pseudogenes that may cause false-positive results. (iv) It may detect not clinically relevant resistance genotypes; (v) Mutations in primer binding sites can generate false-negative results; (vi) It presents low clinical sensitivity when performed directly on mixed microbial samples due to inhibition of nucleic acid amplification or a limited number of targets; (vii) Regulatory mutations that affect gene expression are not detected unless a quantitative measurement of the specific mRNA is targeted; (viii) Unlike for conventional culture-based susceptibility test methods, no standards exist for performing genetic testing methods (Sundsfjord et al., 2004).

Nucleic acid-based technology can be divided into hybridization systems and amplification systems, although most amplification technologies are also partly based on hybridization technology.

In hybridization, the DNA in a sample is rendered single stranded and allowed to combine with a single-stranded probe. Early hybridizations were performed with target DNA immobilized on a nitrocellulose membrane, but nowadays a variety of different solid supports are used. After binding of the target, the probe can hybridize. Probes can be labeled with a variety of reporters, including radioactive isotopes, antigenic substrates, enzymes or chemiluminescent compounds. Current modalities of hybridization DNA or RNA that have been used to detection of antimicrobial resistance in *Staphylococcus* spp. are Southern and Northern Blotting, FISH (Fluorescence *In Situ* Hybridization), microarray and Branched DNA (bDNA).

In Southern blotting, DNA becomes immobilized on a membrane and can be used as a substrate for hybridization analysis with labelled DNA or RNA probes that specifically target individual restriction fragments in the blotted DNA (Southern, 1975). The major difference between Southern and Northern blotting is that in the latter, RNA, rather than DNA, is immobilized in the membrane. The Southern blotting techniques was utilized to detect *mec*A gene in *Staphylococcus aureus* and to evaluate the efficiency of the techniques as PCR (Bignardi et al., 1996; Lan Mo & Qi-nan Wang, 1997).

Fluorescence *In Situ* Hybridization (FISH) is a technique originally developed for clinical diagnosis (Levsky & Singer, 2003). This approach applies the principle of hybridization involving the penetration of a fluorescent labeled sequence-specific nucleic acid probe into fixed cells, followed by specific binding to the complementary sequences of the target nucleic acid. It allows rapid simultaneous detection of structurally intact target genes while they are with the associated organism or particle (Bottari et al., 2006). It involves direct detection of the DNA without amplification of the target sequence and can be especially useful to detect specific bacterial community and antibiotic resistance gene (Rahube & Yost, 2010). A peptide nucleic acid fluorescence in situ hybridization (PNA FISH) (AdvanDx, Woburn, MA, USA) assay was development to rapidly detect *Staphylococcus aureus* (Forrest et al., 2006; Lawson et al., 2011). Peptide nucleic acid (PNA) molecules are pseudopeptides that obey Watson-Crick base-pairing rules for hybridization to complementary nucleic acid targets (RNA and DNA) (Nielsen et al., 1994). Due to their uncharged, neutral backbones, PNA probes exhibit favorable hybridization characteristics such as high specificities, strong affinities, and rapid kinetics, resulting in improved hybridization to highly structured targets such as rRNA. In addition, the relatively hydrophobic character of PNA compared to that of DNA oligonucleotides enables PNA probes to penetrate the hydrophobic cell wall of bacteria following mild fixation conditions that do not lead to disruption of cell morphology (Stefano & Hyldig-Nielsen, 1997).

DNA microarrays are based on the principle of hybridization which allow the mass screening of sequences. The method is based upon gene-specific probes (oligonucleotides or PCR amplicons) deposited on a solid surface like glass or a silicon chip. The test DNA is extracted, labelled and hybridized to the array. Target-probe duplexes are detected with a reporter system. Probe-target hybridization is usually detected and quantified by detection of fluorophore-, silver-, or chemiluminescence-labeled targets to determine relative abundance of nucleic acid sequences in the target (Schena et al., 1995). Microarray technology enables detection of a large number of resistance genes in a single experiment and has the potential for significant automation in a microchip format. However, a cost-effective and user-friendly format for application in antimicrobial susceptibility testing remains to be developed (Sundsfjord et al., 2004). There are many examples of the use of DNA microarray for detection of antibiotic resistance genes in staphylococcal (Cui et al., 2005; Frye et al., 2006; Garneau et al., 2010; Monecke et al., 2007; Zhu et al., 2007a). Recently, a team of scientists at the University of Dublin, the Irish National MRSA Reference Laboratory and the University of Dresden and Alere Technologies in Germany identified a new MRSA strain using high throughput DNA microarray screening. The new strain is not detected as MRSA by routine conventional and real time DNA-based polymerase chain reaction (PCR) assays commonly used to screen patients for MRSA (Shore et al., 2011).

Branched DNA (bDNA) was developed by Chiron Corp. and uses multiple hybridization sites for enzyme-coupled probes (Nolte, 1998). Target-specific probes bound to a solid surface are allowed to capture target ssDNA. A second probe is allowed to hybridize with the target. This probe has a 5′ extension that does not hybridize with the target. This extension can hybridize with a bDNA probe. This probe has a bristle-like structure. At least 15 bristles are attached to each probe, and as many as three alkaline phosphatase reporter molecules can bind to each bristle. A signal is generated by the addition of a chemiluminescent substrate. Branched DNA was used to detect *mecA* gene in *Staphylococcus* spp. culture and from blood (Kolbert et al., 1998; Zheng et al., 1999).

The amplification systems include, but are not limited to, simple and multiplex PCR, PCR-RFLP, PCR-single-strand conformation polymorphism (PCR-SSCP), DNA sequencing and real-time PCR. Amplification methods are more easily adapted in the laboratory compared to DNA probe assays and are the preferred methods for genetic detection of resistance determinants. An internal amplification control for both sample preparation and amplification is recommended to exclude false-negative results using consensus 16S rDNA primers or a more genus-or species-specific target; e.g. the *nuc* gene for *Staphylococcus aureus* (Brakstad et al., 1992; Hoorfar et al., 2004; Vannuffel et al., 1995). It is also critical that negative controls without template DNA and positive controls with defined targets be included to check for false-positive and false-negative results, respectively.

The Polymerase Chain Reaction (PCR) was first described by Mullis et al. (1987), and its first diagnostic application was published by Saiki et al. (1988). The technique became broadly used after the introduction of a thermostable DNA polymerase from *Thermus aquaticus* (Taq DNA polymerase) (Saiki et al., 1988) and the development of automated oligonucleotide synthesis and thermocyclers. PCR involves cycles of heating the sample for denaturing, annealing of the primers, and elongation of the primers. It has been the most commonly used nucleic acid amplification technique in the detection of antimicrobial genes, including *Staphylococcus aureus* (Simeoni et al., 2008).

Multiplex Polymerase Chain Reaction (Multiplex PCR) is a modification of PCR that have also been used to detection of antimicrobial genes in *Staphylococcus aureus* (Amghalia, et al., 2009; Braoios et al., 2009; Zhang et al., 2005) which consists of multiple primer sets within a single PCR mixture to produce amplicons of varying sizes that are specific to different DNA sequences. By targeting multiple genes at once, additional information may be gained from a single test run that otherwise would require several times the reagents and more time to perform.

The specificity of the amplicon can be confirmed by various methods such as restriction fragment length polymorphism (RFLP) analysis, single-strand conformational polymorphism (SSCP) analysis or DNA sequencing.

Restriction Fragment Length Polymorphism, or RFLP is a technique that exploits variations in homologous DNA sequences. It refers to a difference between samples of homologous DNA molecules that come from different locations of restriction enzyme sites. In PCR-RFLP analysis, the PCR product is digested by restriction enzymes and the resulting *restriction* fragments are separated according to their lengths by gel electrophoresis. It has been used to detect *groEL* gene in order to differentiate CNS species (Santos et al., 2007).

In PCR-single-strand conformation polymorphism (PCR-SSCP), the PCR amplication product is denatured into two single-stranded molecules and subjected to nondenaturing polyacrylamide gel electrophoresis. Under nondenaturing conditions, the single-stranded DNA (ssDNA) molecule has a secondary structure that is determined by the nucleotide sequence, buffer conditions, and temperature. PCR-SSCP is capable of detecting more than 90% of all single-nucleotide changes in a 200-nucleotide fragment (Hayashi, 1992).

DNA sequencing is almost universally performed by dideoxysequencing (Sanger et al., 1977) and is a well-known technique. Technological developments brought DNA sequencing within the capabilities of at least some diagnostic laboratories. The latest developments in nucleic acid sequence techniques, the pyrosequencing, have made the detection of mutational resistance easier by rapid DNA sequence analysis (Ronaghi et al., 1998). This technique has been used in the detection of linezolid resistance in enterococci, and to identify point mutations in 23S rRNA genes of linezolid-resistance *Staphylococcus aureus* and *Staphylococcus epidermis* (Sinclair et al., 2003; Zhu et al., 2007b) as well as rapid bacterial identification (Ronaghi et al., 2002).

The laborious post-PCR work and problems with carry-over contamination have been largely removed by the advent of real-time PCR, a powerful improvement on the basic PCR technique (Higuchi et al., 1993). The combination of fluorescent detection strategies with appropriate instrumentation enables a more accurate quantification of nucleic acids. This quantification is achieved by the measure ofthe increase in fluorescence during the exponential phase of PCR. The use of fluorescent agents and probes that only generate a fluorescence signal on binding to their target enables real-time amplification assays to be carried out in sealed tubes, eliminating the risk of carryover contamination. Different techniques are available to monitor real-time amplification. The amplification process can be monitored using nonspecific double-stranded deoxyribonucleic acid (DNA) binding dyes or specific fluorescent hybridization probes.

Four different chemistries, SYBR® Green (Molecular Probes), TaqMan® (Applied Biosystems, Foster City, CA, USA), Molecular Beacons and Scorpions® are available for real-time PCR.

Real-time PCR techniques have permitted the development of routine diagnostic applications for the microbiology laboratory (Espy et al., 2006; Mackay, 2004). Several reports have described the use of these techniques for detection of resistance determinants and surveillance of antimicrobial-resistant *Staphylococcus* spp. (Fang & Hedin, 2003; Huletsky et al., 2004; Palladino et al., 2003; Paule et al., 2005; Thomas et al., 2007; Volkmann et al., 2004). The ability to monitor the accumulating amplicon in real time is based on labelled primers, oligonucleotide probes and/or fluorescing amplicons producing a detectable quantitative signal related to the amount and specificity of the amplicon. Several improvements have been introduced. Reduced amplicon size, shorter cycling times and removal of separate post-PCR detection systems have allowed automation, reduced the detection time, and minimized the risk for carry-over contamination. Other significant technical developments include multiplex PCR assays using more than one primer set for simultaneous detection of several antimicrobial resistance genes (Depardieu et al., 2004; Martineau et al., 2000a; Sabet et al., 2006; Šeputienė et al., 2010; Suhaili et al., 2009).

4.1 Molecular Identification/Typing of *Staphylococcus* species

Earlier studies on the taxonomy of *Staphylococcus* species based on DNA-DNA reassociation indicated that in the genus there were nine distinct species groups, represented by *S. epidermidis*, *S. saprophyticus*, *S. simulans*, *S. intermedius*, *S. hyicus*, *S. sciuri*, *S. auricularis*, S. *aureus*, and *Staphylococcus caseolyticus* (Kloos & George, 1991). Several molecular targets have been exploited for the molecular identification of *Staphylococcus* species, including the 16S rRNA gene (De Buyser et al., 1992), the tRNA gene intergenic spacer (Maes et al., 1997), the heat shock protein 60 (HSP60) gene (Kwok et al., 1999), and the *femA* gene (Vannuffel et al., 1999). These targets, however, have been exploited through the technology of molecular probe hybridization, and therefore, they are useful only in laboratories that have the complete panel of probes and then only for identifying recognized *Staphylococcus* species. Further molecular targets that have been identified include the *nuc* gene, which occurs only in *S. aureus* (Brasktad et al., 1992), and a chromosomal DNA fragment specific for *Staphylococcus epidermidis* (Martineau et al., 1996).

The *rpoB*, gene encoding the highly conserved β subunit of the bacterial RNA polymerase, has previously been demonstrated to be a suitable target on which to base the identification of enteric bacteria (Mollet et al.,1997), spirochetes (Renesto et al.,2000), bartonellas (Renesto et al.,2001), and rickettsias (Drancourt & Raoult, 1999). The gene has been shown to be more discriminative than the 16S ribosomal DNA (rDNA) gene, which has also been used for identifying staphylococal bacteria (Mollet et al., 1997). In contrast to the probe hybridization technique and the RFLP approach, sequencing enables any isolate to be characterized, including new species by their phylogenetic relationships.

Other suitable targets for the molecular identification of *Staphylococcus* species that have been proposed include the *femA* gene, which was used in a multiplex PCR-reverse hybridization approach to identify 55 clinical isolates (Vannuffel et al.,1999). These, however, included only five *Staphylococcus* species, namely *S. aureus*, *S. epidermidis*, *S. hominis*, *S. saprophyticus*, and *S. simulans*. Finally, molecular identification methods for the identification of one or only a few *Staphylococcus* species have been reported for *S. saprophyticus* (Martineau et al., 2000b), *S. aureus* (Benito et al., 2000), and *S. epidermidis* (Wieser & Busse, 2000). Whole-genome DNA-DNA hybridization analysis (Svec et al., 2004) allows species identification, but the method is not suitable for routine use.

Accurate and rapid typing of *Staphylococcus aureus* is crucial to the control of infectious organisms (Naffa et al., 2005), and many different pheno- and genotyping methods have been used to distinguish their strains. Typing methods should have high and relevant discriminatory power and typeability, good reproducibility, applicability to all organisms of interest, ease of use, portability and low cost (Struelens et al., 1996). The common phenotyping techniques used for discriminating between bacteria from a single species are serotype, biotype, bacteriophage typing, or antibiogram. Techniques DNA-based such as pulsed field gel electrophoresis (PFGE), amplified fragment length polymorphism (AFLP), and multilocus sequence typing (MLST) have been many used (Melles et al., 2004; Murchan et al., 2003; Tenover et al., 1994). Other common techniques use the Polymerase Chain Reaction (PCR) targeted to specific sequences, for example ERIC-PCR; the resulting reactions yield fragments of different sizes, which can be used to discriminate between bacterial types. Sequencing an entire bacterial genome, and, using micro-array technologies,

comparing strains to a reference strain (comparative genomic hybridization) is now technically feasible; however, the cost and time required limits the applicability for most epidemiologic studies (Foxman et al., 2005).

PFGE is the most commonly used method when studying local or short-term *S. aureus* epidemiology (Chung et al., 2000). PFGE involves embedding organisms in agarose, lysing the organisms in situ, and digesting the chromosomal DNA with restriction endonucleases that cleave infrequently (Finey, 1993; Goering & Winters, 1992). Slices of agarose containing the chromosomal DNA fragments are inserted into the wells of an agarose gel, and the restriction fragments are resolved into a pattern of discrete bands in the gel by an apparatus that switches the direction of current according to a predetermined pattern. The DNA restriction patterns of the isolates are then compared with one another to determine their relatedness. Multicentre studies using PFGE are now possible due to recent advances in the standardization of electrophoresis conditions (Chung et al., 2000; Oliveira et al., 2001) and the development of normalization and analysis software (Duck et al., 2003). Interpretative criteria for use in comparing complex PFGE patterns in outbreaks have been applied to non-outbreak situations to track the national and international dissemination of *S. aureus* clones (Tenover et al., 1995). The use of PFGE typing with adjusted interpretation criteria for grouping patterns with < 7 bands difference has been shown to correspond to clonal assignments made by other methods (Denis et al., 2004). The main criticisms of this technique for *S. aureus* are that PFGE may, on occasion, be too discriminatory for other than local or short-term epidemiological analyses, the arbitrary nature of the interpretive criteria used and the requirement for occasional subjective analysis of complex band patterns (Murchan et al., 2004).

Amplified fragment length polymorphism (AFLP) (Vos et al., 1995), a typing method, also documents the contribution of "accessory genetic elements" next to genome–core polymorphisms. AFLP scans for polymorphism in actual restriction sites and the nucleotides bordering these sites. As such it documents nucleotide sequence variation, insertions and deletions across entire genomes (Vos et al., 1995).

Multilocus sequence typing (MLST) (Maiden et al., 1998) has had a large impact on the field of bacterial typing and it has been used as an investigatory tool in many studies of *S. aureus* evolution and epidemiology (Aires de Sousa et al., 2003, Coombs et al., 2004, Enright et al., 2002; Mato et al., 2004). MLST characterizes bacterial isolates on the basis of sequence polymorphism within internal fragments of seven housekeeping genes, representing the stable "core" of the staphylococcal genome. Each gene fragment is translated into a distinct allele, and each isolate is classified as a sequence type (ST) by the combination of alleles of the seven housekeeping loci (Enright et al., 2002). MLST has a major advantage over PFGE as a reference method due to the unambiguous nature of DNA sequences which can be stored easily along with corresponding clinical information on each isolate in internet-linked databases. The *S. aureus* MLST website (www.mlst.net) currently contains information on > 1500 isolates from humans and animals from 40 different countries and represents a useful global resource for the study of the epidemiology of this species and the surveillance of hyper-virulent and / or antibiotic resistant clones.

The variety of molecular techniques used for diagnostic applications demonstrate that no universal technique exists which is optimal for detection of nucleic acids. The choice of a particular technique is also dependent on the information required or the targets under

consideration, but some techniques are more favored than others. Hence, the genetic approach based on today's test principles cannot substitute for phenotypic methods in routine antimicrobial susceptibility testing. Novel resistance mechanisms will arise continuously or unknown pre-existing resistance genes will be mobilized from environmental reservoirs and spread under antimicrobial selection (Barlow et al., 2004). Thus, the role of traditional susceptibility testing will continue to be important. Rather the rationale for genetic assays is to complement conventional phenotypic analyses (Sundsfjord et al., 2004). Challenges that remain include the variety of point mutations or genes leading to resistance and the labor-intensive nature of current amplification methods. DNA chip technology combined with automated amplification techniques has the potential to meet these challenges. However, the development of DNA chips containing a broad range of resistance markers that are usable for many different species remains a formidable challenge and requires a broader knowledge of resistance markers than is currently available (Sundsfjord et al., 2004).

5. The relevance of surveillance for the prediction of antibiotic resistance

This topic discusses the relevance and limitations of surveillance initiatives in veterinary practice. Antibiotic resistance surveillance is based on the identification of new challenges, detection of new resistance mechanisms, monitoring the impact of new empiric antibiotic prescribing, identification of outbreaks of resistant organisms, detection of bacterial misidentification and promotion of the establishment of standards and **guidelines** for **education** and training for veterinaries, animal keepers, animal owners and the general public.

Cats and dogs represent potential sources of spread of antimicrobial resistance due to the extensive use of antimicrobial agents in these animals and their close contact with humans. Modern society has contributed to radical changes in the relationship between companion animals and humans through the years, with a significant raise in cats and dogs population and to a closer contact with humans (Guardabassi et al., 2004).

The introduction of a new drug, especially an antibiotic, has to be monitored in order to achieve its real benefit to the target audience. Recently, azithromycin was introduced to Brazilian pet market claiming to be an advantageous antimicrobial alternative to dogs and cats infections such as pyodermitis, external otitis, respiratory and urinary tract disturbs. Azithromycin have a remarkable application due to its superior pharmacokinetics properties and broad spectrum activity, including Gram-positive and negative bacteria species, intracellular pathogens and protozoan parasites. Pereira et al. (2009) evaluated the resistance to azithromycin of 225 clinical samples from different infectious sites of pet animals in order to establish the benefits of introducing this drug in veterinary therapy in Brazil since it has already been used for human therapy. Azithromycin resistance can be caused by several mechanisms, such as target modification mediated by a 23S rRNA methylase, presence of efflux pumps and drug inactivation (Lim et al., 2002). These resistance mechanisms were identified in a wide range of Gram-positive and negative bacteria, such as, *Staphylococcus* spp, *Streptococcus* spp, *Enterococcus faecium, Corynebacterium* spp, *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacteroides* spp, all of them implicated in the etiology of household pets infections. Among them, *Staphylococcus* spp, a resident member of the normal cutaneous and mucosal microbiota of humans and animals, stands out as an

important pathogen involved in several animals infectious diseases due to its wide range of virulence factors and ability to overcome antimicrobial effects (Garber, 2001). Predominant staphylococci azithromycin resistance mechanisms are that mediated to *erm*(A) and *erm*(C) determinants of 23S rRNA methylase and *mef* genes that encode efflux pumps. The *erm*(A) genes are mostly spread in methicillin-resistant strains and are borne by transposons related to Tn*554,* whereas *erm*(C) genes are mostly responsible for macrolide resistance in methicillin-susceptible strains and are borne by plasmids (Lim et al., 2002). Most of the knowledge applied to define microbiologic use and dose of azithromycin was based on *Staphylococcus aureus* assays, because this specie acts as a classical pathogen of human infections, but the frame of etiology and antimicrobial susceptibility pattern change when animal pathogens are considered.

Otherwise, gentamicin, even being the most utilized antimicrobial in bases for intramammary use, keeps its effectiveness against staphylococci isolated from mastitic milk. Those data support the idea of the importance of monitoring the impact of new/old empiric antibiotic prescribing

6. Concluding remarks

- *Staphylococcus* species distribution considers that different animal species have a different staphylococcal microbiota divided into coagulase-positive and coagulase-negative groups. In veterinary medicine, besides *S. aureus*, others coagulase-positive species are reported as important pathogens, such as *Staphylococcus intermedius*, the new specie *S. pseudintermedius*, *S. delphini*, *S. schleiferi* subsp. *coagulans* and *S. aureus* subsp. *anaerobius*. Several Coagulase-negative Staphylococci have been isolated from animal clinical specimens such as *Staphylococcus epidermidis*, *Staphylococcus simulans*, *Staphylococcus xylosus*, *Staphylococcus chromogenes*, *Staphylococcus warneri*, *Staphylococcus haemolyticus*, *Staphylococcus sciuri*, *Staphylococcus saprophyticus*, *Staphylococcus hominis, Staphylococcus caprae, Staphylococcus cohnii* subsp. *cohnii*, *Staphylococcus cohnii* subsp. *urealyticus*, *Staphylococcus capitis* subsp. *capitis* and *Staphylococcus capitis* subsp. *urealyticus*.
- *Staphylococcus aureus* is considered the most pathogenic specie, especially due to its ability to produce a large range of virulence factors that enables it to colonize different tissues of a large range of animal species, such as coagulase, slime, protein A, hemolysins. Otherwise, like *S. aureus*, the *S. intermedius* strains isolated from animals have been reported to produce an array of virulence factors, including leukotoxin, enterotoxin, and hemolysins, together with elements essential for biofilm formation.
- The epidemiological and clinical importance of Staphylococcal species is not only because of its distribution and pathogenicity but especially due to its ability to overcome antimicrobial effects. So there is a need for continued vigilance and systematic study to enlarge the understanding of its dynamic. Considering the spread of MRSA strains it is necessary to determine risk factors for animal infections, especially for household pets that live in strict contact to men, the relationship between animal and human carriage, and the genetic relationship of animal and human strains.
- hVISA or VISA phenotype, mostly MRSA, have now been reported for many countries and it is considered to be related to the bacterial cell wall thickening, a passive resistance mechanism that reduces vancomycin access to its active site. Also MRSA strains that are also VRSA have been reported in the United States. VRSA phenotype is

due to acquisition from *Enterococcus* spp. of the *vanA* operon, carried by transposon Tn1546, resulting in high-level resistance. The emergence of enterococci vancomicin-resistant strains has been related to the use of avoparcin as growth promoter in swine culture and it seemed to arise in staphylococci due to the transfer of glycopeptide- and macrolide-resistance genes by transconjugation among enterococci and from *Enterococcus faecalis* to *S. aureus.*

- The large range of molecular techniques available for use demonstrates that no universal technique exists which is optimal for detection of nucleic acids. The choice of a particular technique is also dependent on the information required or the targets under consideration. Hence, the genetic approach based on today's test principles cannot substitute for phenotypic methods in routine identification and antimicrobial susceptibility testing.
- Antibiotic resistance surveillance is based on the identification of new challenges, detection of new resistance mechanisms, monitoring the impact of new empiric antibiotic prescribing, identification of outbreaks of resistant organisms, detection of bacterial misidentification and promotion of the establishment of standards and **guidelines** for **education** and training for veterinaries, animal keepers, animal owners and the general public.

7. Future challenges

Tenover (2008) in his article "Vancomicin-resistant *Staphylococcus aureus*: a Perfect but Geographically Limited Storm?" gives us a clue that antibitioc resistance issue is not so simple answer. Science is a creative activity that request exploration and gambling. We have to be open-minded to understand that some evolutionary steps are more successful than others and the pathways to resistance are not so predictable. As Tenover (2008) said: "Predicting which resistant strains will ultimately survive and disseminate is virtually impossible; predicting that at least some strains will disseminate broadly is a certainty." The biggest challenge is keep researching in order to enhance our knowledge of the mechanisms beyond resistance, the evolutionary pathways of resistance among microorganisms, and selective pressure factors that contribute to the expression of underlying genes.

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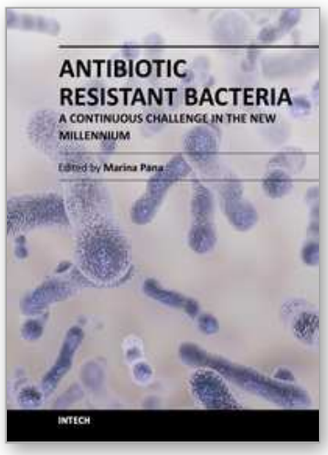

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Antibiotic-resistant bacterial strains remain a major global threat, despite the prevention, diagnosis and antibiotherapy, which have improved considerably. In this thematic issue, the scientists present their results of accomplished studies, in order to provide an updated overview of scientific information and also, to exchange views on new strategies for interventions in antibiotic-resistant bacterial strains cases and outbreaks. As a consequence, the recently developed techniques in this field will contribute to a considerable progress in medical research.

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