Antimicrobial resistance in animal production: an overview*

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ABSTRACT. de Souza M.M.S., Coelho S.deM.deO., Coelho I.daS., Soares B.daS., da Motta C.C., de Melo D.A., Dubenczuk F.C., Santiago, G.S., Pimenta R.L., Marques V.F. & Alencar T.A. **Antimicrobial Resistance in Animal Production: an Overview.** [Resistência antimicrobiana em animais de produção: uma visão geral]. *Revista Brasileira de Medicina Veterinária, 38(supl. 3):68-74, 2016.* Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, BR 465, Km 7, Seropédica, RJ, 23890-000, Brasil. E-mail: milianemss@gmail.com

In the last two decades, antimicrobial resistance emerged as a puzzling multifactorial challenge. The role of the use of antimicrobials as growth promoters in animal production exerting a positive selection pressure which can favor the survival of resistant strains over susceptible ones and the spread of resistance genes is the subject of several researches throughout the world. Otherwise, defenders of the growth promoter use argue that antimicrobial abolishment will result in several disorders in production chain. Some researches support the idea that there are efficient and viable alternatives such as probiotics, enzymatic complex and natural products that can contributes to growth promotion without enhance antimicrobial resistance. Considering the importance of animal production to the social and economic development of Brazilian society and the crucially of this theme, this review presents some features of antimicrobial resistance in dairy milk and poultry industries, two major production systems. Also, it discusses the challenges faced by researchers in the establishment of an accurate resistance profile taking in account three important features: species identification, species diversity and virulence factors that may contribute to the observed resistance pattern, and additionally, the emergent role of Enterobacteriaceae in antimicrobial resistance in animal production environment.

KEY WORDS. Antimicrobial resistance, growth promoters, poultry, dairy milk.

RESUMO. Nas últimas duas décadas, a resistência antimicrobiana emergiu como um intrigante desafio multifatorial. O papel do uso de antimicrobianos como promotores de crescimento em produção animal, exercendo uma pressão de seleção positiva que pode favorecer a sobrevivência de cepas resis-

tentes sobre as sensiveis, é o tema de vários estudos em todo mundo. Por outro lado, defensores do uso de promotores de crescimento argumentam que abolir os antimicrobianos resultará em vários desequilibrios na cadeia produtiva. Alguns estudos sustentam a ideia de que existem alternativas viáveis e eficientes tais como probióticos, complexos enzimáticos e produtos naturais que podem contribuir para a promoção do crescimento sem incrementar a resistência antimicrobiana. Considerando a importância da produção animal para o desenvolvimento econômico e social da sociedade Brasileira e a crucialidade deste tem, esta revisão apresenta alguns aspectos da resistência antimicrobiana na indústria avícola e leiteira, dois maiores sistemas de produção. Também, discute os desafios enfrentados pelos pesquisadores no estabelecimento de um perfil de resistência que leva em conta três importantes aspectos: identificação de espécies, diversidade das espécies e fatores de virulência que pode contribuir para o padrão de resistência observado, e adicionalmente, o papel emergente das Enterobacteriaceae na resistência antimicrobial no ambiente da produção animal.

PALAVRAS-CHAVE. Resistência antimicrobiana, promotores de crescimento, indústria avícola e indústria leiteira.

INTRODUCTION

In the last two decades, antimicrobial resistance emerged as a puzzling multifactorial challenge (Howard et al. 2014, Howard et al. 2015). Indeed, more than 700 000 people are estimated to die each year because of multidrug-resistant bacteria (David 2016). Nowadays, it is accepted that the use of antimicrobials in therapeutic levels for health care or sub therapeutic levels as growth promoters in animal production influences the increasing of this resistance due to the exertion of a selection pressure which can favor the survival of resistant strains over susceptible ones and the spread of resistance genes.

In Brazi,l a wide variety of growth promoters was authorized by the Ministry of Agriculture (MAPA) and largely used in animal production. Defenders of this model argued that antimicrobial abolishment will result in higher morbidity, with a consequent raise of antimicrobial therapeutic use and consequently higher mortality. Also that it will directly implicate the efficiency of productivity as animals without growth promoters have a higher food consumption to achieve the same weight gain (Cervantes 2012). Since the beginning of this century, MAPA instituted work groups in order to analyze and evaluate the use of substances as animal feed additives products. Some active principles were banned for the purpose of growth promoters. The MAPA issued Order n. 31 banning the use of arsenical and antimonial products (Brazil 2002). Chloramphenicol and nitrofurans were banned in 2003 by Normative Ruling n. 09 (Brazil 2003). Amphenicols, tetracyclines, beta-lactams (benzyl-penicillins and cephalosporins), quinolones, and systemic sulfonamides were banned for that purpose by Normative Ruling n. 26 (Brazil 2009). In 2012, spiramycin and erythromycin were also banned by Normative Ruling n. 14 (Brazil 2012). Nevertheless it looks like a long and difficult pathway to go through.

On the other hand, the European Economic Community established severe restrictions for products presenting antimicrobial residues defending the idea that this subtherapeutic use contributes to a positive selective pressure and to the spread of antimicrobial resistance genes between different pathogens and must be strongly discouraged. 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* seem to be related to the adoption of these restrictive measures (Aarestrup et al. 1996)

Some researches supports the idea that there are efficient and viable alternatives growth promoters to the antimicrobial use such as probiotics, enzymatic complex and natural products like cinnamon, garlic and oregano (Dalólio et al. 2015, Dias et al. 2015). 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. Enzimatic complex enhances feed digestibility, especially for those feed which have greater fiber content (Park et al. 2016). As a consequence it contributes to growth promotion without enhance antimicrobial resistance.

Nowadays new approaches in genomic technology, seems to be the key for some answers in the comprehension of antimicrobial resistance spread. The advantages of Whole Genome Sequence-based

typing methods include inferring the most probable phylogenetic lineages of descent between isolates to infer the direction and route of pathogen transmission, from environmental, animal or human sources and reservoirs and the in silico prediction of acquired antimicrobial resistance mechanisms, pathogenicity and virulence determinants as well as correlates of epidemiological/ecological fitness associated with epidemic spread, also described as *high-risk clones*. It is expected that in a close future the establishment of standards and systems enabling the wide use of WGS as the method of choice for typing microbial pathogens will improve pathogen detection, identification, antimicrobial resistance profiling and biological risk prediction (ECDC 2016).

ANTIMICROBIAL RESISTANCE IN DAIRY INDUSTRY

Dairy industry performs a very social important activity that yielded more than three million jobs in the last decade. Brazil produced 35.2 billion of liters of milk in 2014. The South region that figured out as the highest producer accounted for 12.2 billion. Nevertheless, Minas Gerais is the main milk producing state in Brazil. Otherwise, Rio de Janeiro produced only 540 million of liters of milk, a reduction of 5.1% considering 2013. Mastitis, a multifactorial inflammation of the mammary gland is one of the most important economic losses causes in milk production and the incorporation of adequate hygienic procedures to daily handling is considered the most efficient way to control it. Otherwise the indiscriminate use of antimicrobials is a negative feature in this control, raising the positive pressure selection and favoring the spread of resistance genes. An epidemiologic inquiry and bacteriological survey performed in dairy farms located at South Fluminense Dairy Belt in the State of Rio de Janeiro showed that 80% of studied dairy properties presented unsatisfactory hygienic conditions in milking line and water supply so mastitis control relies frequently on antimicrobial overuse (Alencar et al. 2014).

Mastitis is differentiated based on clinical and epidemiological features. It can be classified as clinical, once presenting signals such as swelling, redness and painful touch, or subclinical, when inflammation presents no visible signals and can only be detected by means of specific tests as California Mastitis Test (CMT). Also it can have or not an infectious origin. Infectious mastitis can be caused by several microorganisms, bacteria being the prevalent ones. Depending on the bacterial species implicated on its etiology it is classified as contagious or environmental mastitis. *Staphylococcus aureus* and *Streptococcus agalactiae* are the most important agents of contagious form. Nevertheless, others coagulase positive and negative staphylococci have also been implicated in this form. Environmental mastitis is due to the presence of agents related to unhealthy hygienic conditions such as Escherichia coli (Santiago et al., 2015, Coelho et al. 2011).

ANTIMICROBIAL RESISTANCE IN POULTRY INDUSTRY

Brazil figures among the greatest poultry meat producers in the world for the last two decades. The South region is the highest producer and exporter. (UBABEF 2011, 2012). Several factors have raised Brazil to a privileged position in the chicken meat production and exports markets. The productivity of this industry is supported by the use of modern management and nutritional practices, genetic improvement, modern facilities, and the integrated production system, which was largely stimulated by the partnership between processing companies and farmers, resulting in high quality products and low production costs (Brasil 2012). Also it must be taken in account factors such as the abundance of land, fertile soil to produce feedstuff, favorable climate, and innovativeness of the companies in overcoming challenges (Nääs et al. 2015). The most significant challenges of poultry industry are to lowering the costs of production and increase productivity. This contributes to the higher population density and consequently to infectious diseases spread and leads to the constant need of pathogen prevention and control so the focus on poultry health was particularly important for the development of the poultry industry. In this context, antimicrobials are frequently overused. Although there is no consensus on the degree of influence of the use of antimicrobials in animals on the emergence and dissemination of bacteria resistant to antimicrobials in humans, experimental evidences and epidemiological and molecular studies point out a relationship between the use of antimicrobials and the emergence of resistant bacterial strains in animals and their dissemination to humans, especially through the food chain (Gouvêa 2015).

ANTIMICROBIAL RESISTANCE IN DAIRY MILK AND POULTRY MAJOR PATHOGENS

Staphylococcus spp and the beta-lactamic resistance

Staphylococcus spp. is the **most commonly isolated agent** in bovine mastitis, especially considering *Staphylococcus aureus* (Coelho et al. 2009). Its importance is not only because of its distribution and pathogenicity but also due to its ability to overcome antimicrobial effects. Its high antimicrobial resistance level, especially to beta-lactams, favors treatment failures and its persistence in herd environment. Bacterial resistance mechanisms to this antimicrobial class include production of ß-lactamases and low-affinity penicillin-binding protein 2a (PBP2a) determined by the presence of the chromosomal genes *bla* and *mec*A, respectively. The latter, involved in oxacillin-resistance, precludes human therapy with any of the currently available ß-lactam antibiotics, and is considered to predict resistance to several classes of antibiotics (Moon et al. 2007). Once phenotypic expression of beta-lactam resistance in *Staphylococcus* isolates is usually heterogeneous, the amplification of *mec*A gene is prescribed as a gold standard in the detection of this resistance (CLSI 2014).

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Otherwise, our research results in animal science field concerning to bovine staphylococci beta-lactam resistance does not support that *mec*A gene plays the same significant role in the detection of this resistance. As a matter of fact, our previous studies reported several phenotypically oxacillin-resistant isolates that tested negative for *mec*A gene (Coelho et al. 2009, Mendonça et al. 2012, Soares et al. 2012).

Theories have been proposed to explain the lack of correlation between the observed phenotypical methicillin-resistance and the detection of *mec*A gene. *Staphylococcus aureus* antimicrobial resistance, especially to beta-lactams, favors treatment failures and its persistence in herd environment. To improve the accuracy of beta-lactamic detection we developed a more specific primer for *mec*A gene detection based on the comparison of the conserved regions from distinct host origins and also investigated the presence of homologue *mec*A$_{L-GA251}$ in bovine strains. For *mec*A gene PCR, new primers were designed based on the sequences of human *S. aureus* (HE681097) and bovine *S. sciuri* (AY820253) *mec*A. The new primers based on the *S. aureus mec*A sequence amplified fragments of human and equine CNS and the ones based on *S. sciuri mec*A sequence only yielded fragments for *S. aureus* bovine strains. Multiples alignments of *mec*A gene sequences from bovine, human and equine revealed punctual but significant differences in bovine strains that can lead to the *mec*A gene detection impairment. The observed divergences of *mec*A gene sequences are not a matter of animal or human origin, it is a specificity of bovine samples (Melo et al. 2014).

CHALLENGES IN PREDICTING ANTIMICROBIAL RESISTANCE IN STAPHYLOCOCCI ISOLATES OF ANIMAL ORIGIN

The assessment of antimicrobial resistance in bacterial isolates from different animal sources is a defiant task once a significant number of variants must be taken in account. The development of a high-accuracy methodology must consider three features: species identification, species diversity and virulence factors that may contribute to the established resistance pattern.

First of all, the species identification is a step of utmost importance due to the great variety of bacterial species in animal environment and the potential hazard of a misidentification to the evaluation of antimicrobial resistance. The best example is genus *Staphylococcus* spp. In Veterinary Medicine, others coagulase-positive staphylococci than *S. aureus* are reported as important pathogens, such as *S. 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*. Besides SIG, others significant coagulase-positive or coagulase-variable in animals are *S. schleiferi* subsp. *coagulans, S. aureus* subsp. a*naerobius*, *S. hyicus*, *S. lutrae*, e *S. agnetis* (Souza et al. 2012). Besides *S. aureus*, species such as *S. hyicus* and *S. intermedius* are implicated in bovine mastitis etiology.

These species are often misdiagnosed as *S. aureus*. Several currently available methods for the identification of *Staphylococcus* spp., including molecular techniques, are widely used worldwide. Recently, matrix-assisted laser desorption ionization - time-offlight mass spectrometry (MALDI-TOF-MS) has been attracting attention for its fast and precise identification of several microorganisms at the species level. Motta et al. (2014) performed a study to evaluate the efficiency of a protocol for *S. aureus* characterization using PCR and M-PCR procedures. MALDI-TOF was considered the gold standard test to evaluate the sensitivity and specificity of the proposed identification protocol. Seventy-two *Staphylococcus* spp., isolates were evaluated. All samples were submitted to PCR for *coa*, *nuc* and 23S rDNA. Out of 33 isolates, genotypically characterized as *S. aureus* and confirmed by MALDI-TOF MS, 2 (6.1%) tested negative for coagulase production. Three isolates were identified as *S. hyicus* (2) and *S. intermedius* (1) by MALDI-TOF MS. The proposed molecular identification schedule achieved 100% sensitivity and specificity as compared to MALDI-TOF MS. This achieved accuracy in *Staphylococcus* spp. identification is crucial for the correct detection of resistance pattern considering the CLSI VET01-S2 (2013) standards that established specific guidelines and cut off for the different staphylococci species.

Staphylococcus species carries genetic heterogeneity and a population characterized by genetically diverse strains. The analysis of the genetic variation appear as an important tool for epidemiological studies. Soares et al. (2017) studied the virulence profile plus *agr* and *spa* typing of 53 strains of *S. aureus* from bovine mastitis. It was possible to establish 18 different profiles. As a matter of fact, it was observed extensive genetic heterogeneity of the selected isolates and no clones the final sample was set in 17 isolates that served to MLST assays, detection of phenotypic biofilm production, detection of antimicrobial susceptibility and genotypic MRSA strains. The typing technique MLST generated five different types of ST/CC, been ST/CC 126 the most prevalent (64.7% - 11/17). Phenotypic analysis of biofilm production, demonstrated a prevalence of positive isolates to the biofilm production genes, however, with a low phenotypic expression of the characteristic. The isolates tested were sensitive to all antibiotics tested except ciprofloxacin, erythromycin and penicillin. It was not possible to detect the presence of any MRSA strain in this study.

Among the factors that contribute to the *Staphylococcus* spp. invasion (Saei 2012), the production of slime, an extracellular mucopolysaccharide, seems to play a crucial role in the adhesion and colonization of the microorganism to the mammary glandular epithelium, favoring the formation of biofilm, their extracellular persistence and ensuring success in their installation and maintenance in the host tissues (Coelho et al. 2011). Biofilm production in *S. aureus* from mastitis can also be associated with antimicrobial resistance (Cucarella et al. 2004). Among the mechanisms responsible for this resistance are the physical and chemical diffusion barrier formed

by the exopolysaccharide matrix, which hinders the penetration of the antimicrobials, the existence of microenvironments that antagonize the antibiotic action, the activation of stress responses that cause changes in bacterial physiology, the stable and slower growth of these microorganisms due to nutrient limitation and also due to the absence of antimicrobial targets (Coelho et al. 2011).

Marques et al. (2017) performed assays to detect the phenotypic expression of biofilm and the presence of structural and regulatory genes involved in the production of this virulence factor. Also pheno-genotypic resistance to beta-lactamic and its possible relation to biofilm production were evaluated. The MIC and MBC for cefoxitin were analyzed for distincts S. aureus isolates. The stronger biofilm producer N-341 presented the highest MIC and MBC (1µg/mL and 64µg/mL, respectively), followed by the moderate producer N-365 (<0.25 µg/mL and 4 µg/mL, respectively). The weaker biofilm producer N-354 also presented the lowest MIC and MBC (<0.25 µg/mL for both). Since N-341 was negative for *bla*Z and *mec*A genes, the high cefoxitin MBC observed must be associated with biofilm protection. Wells et al. (2011) discussed that there is no universal acceptable methodology for antimicrobial resistance and biofilm production study. Based on this it seems that exopolysaccharide (EPS) secreted by the bacteria can act as a barrier that may play a role in this resistance, preventing the adsorption and penetration of antimicrobials. Moreover, the EPS matrix could neutralize or bind these compounds, promoting their dilution to subinhibitory concentrations before they reach the cells. Another factor is that biofilms are composed of dormant and active cell subpopulations, and this difference in bacterial physiology can also influence the efficacy of antibiotics (Raza et al. 2013). These data suggest that a greater understanding of biofilm formation may add to our knowledge concerning bacterial resistance *in vivo*. Thus, studies that uncover colonization factors in biofilm formation are important and will be the basis for the development of treatments for bacterial resistance in biofilm.

THE EMERGENT ROLE OF ENTEROBACTERIACEAE IN ANTIMICROBIAL RESISTANCE IN ANIMAL PRODUCTION ENVIRONMENT

Enterobacteriaceae are the main etiological agents of many animal infections as bovine mastitis and avian colibacillosis usually associated to insufficient hygienic conditions in the animal production like manure, urine and mud present in the environment (Liebana et al. 2013).

They are often resistant to antimicrobials, especially to β-lactam antibiotics due to the production of β-lactamase. Innumerable enterobacteria can produce broad-spectrum β-lactamase (ESBLs) such as AmpC, TEM, SHV, CTX-M and carbapenemase that can be classified into several groups according to the molecular structure and the acting substrate. In addition, they may expand resistance to other antimicrobials that are often used in the animal treatment of infections caused by enterobacteria (Kateregga et al. 2015).

These enzyme producing are believed to be widely distributed among humans and animals. Bacteria producing extended-spectrum β-lactamases (ESBL), already widely studied in clinical microbiology, can be retrieved from cow's milk, beef, pork and chicken, as well as animal feces; however, to date, there are only a few studies concerning their dissemination in animal production environment (Li et al. 2008, Locatelli et al. 2009, Dahmen et al. 2013). In study development by Santiago et al. (2015) with 42 enterobacteria from 381 milk samples obtained from mastitic cows from farms located in the Rio de Janeiro-RJ, the AmpC production was prevalent (47,6%) and the worrying thing is that 30% of them were classified as inducible type. Enterobacteriaceae producing AmpC is reported worldwide and has great clinical importance due to restriction in infections treatment. They also found bla_{TEM}, bla_{SHV}, bla_{CTX-M} and bla_{ampC} genes in 61.9%, 42.8%, 23.8%, 9.5% of isolates, respectively. This genotypic variability detected indicates multiple mechanisms of antimicrobial resistance which is worrying due of the resistance bacterial spread determining a clinical and epidemiological impact for veterinary medicine (Santiago et al. 2015). The possible impact of healthy animals as a potential reservoir for resistance genes should be monitored to prevent the spread of this resistance and to choose suitable prophylactic and therapeutic measures.

In poultry farming the reality does not differ. Unfortunately the use of antimicrobials is widespread in the world's poultry industry, concentrating its utilization in countries holding large herds. In 2010, the total utilization of antimicrobials in animals was 63,151 tons and the projection for 2030 is an 67% increase in consumption, with the most consumed by pig farming followed by poultry (European Medicines Agency 2013). The treatment with antibiotics and also the use of subdoses in animal feed for prophylactic purposes are strongly responsible for the selection of increasingly resistant bacteria. The importance of the emergence of resistant bacteria has been growing considerably and many strains of enteric pathogens multidrug resistant are recognized to have origin in animal reservoir, where they probably have undergone selection when antimicrobial agents are administered for therapy or in doses as growth promoters (Ramchandani et al. 2005). In addition, these growth promoters are poorly absorbed by the intestine and the drug is frequently excreted with feces contaminating the poultry litter. In the final of broiler cycle production, the litter could be use without appropriate treatment on planting vegetable and this situation could spread antibiotic and resistance genes on environment contaminating the food chain.

Despite the ban on the use of β-lactam antimicrobials as growth promoters or prophylactically, their use in therapeutic form is released and the use without adequate technical monitoring is frequent, where rural workers often add the drug to water without knowing their func-

tion, recommended dose and grace period (Brazil 2009). With the purpose of understanding the dissemination of resistant Gram negative in poultry production environment, our study group collected 120 swabs from cloaca and trachea from 60 animals raised on a farm placed in the mountain region of Rio de Janeiro, in 2015 and 2016. *Escherichia coli* and *Bordetella* spp. presented a very evident resistance to amoxicillin+clavulanate and cefoxitin. *E.coli* was characterized as ESBL, AmpC and co-producer of both enzimes in 23%, 4% and 10%, respectively. The classification of β-lactamases in *Bordetella* spp. is not completely clear and defined but we detected phenotype of β-lactamase production in all isolates including resistance to ceftazidime. The intensive use of these antimicrobial agents determined frequently the contamination of food animal production with antimicrobial resistant Gram negative strains. The use of antibiotic such as aminoglycosides, fluoroquinolones, and third- and fourth-generation cephalosporins may lead to the treatment failure and can have serious consequences also for the human patient. ESBL producing microorganisms represent a significant public health problem in several countries and further research and the implementation of infection control measures in veterinary hospitals and clinics, as well as in farms and parks, are necessary to reduce the spread of these resistant bacteria (Beninati et al. 2015).

CONCLUDING REMARKS

Antimicrobial resistance in animal production system is an issue that deserves to be deeply investigated. Considering the pros and cons of the use of antimicrobial as growth promoters is the key to understand its direct impact and secondary effects on animals, human and environment. Although it is not an easy question to answer, the prediction of bacterial resistance is a challenge that must be faced due to the need to improve control measurements that must link the species identification, the species diversity and the virulence factors that may contribute to the established resistance pattern. It is expected that the use of technology will improve pathogen detection, identification, antimicrobial resistance profiling and biological risk prediction.

REFERENCES

Aarestrup F.M., Ahrens P., Madsen M., Pallesen L.V., Poulsen R.L. & Westh H. Glycopeptide susceptibility among Danish *Enterococcus faecium* and *Enterococcus faecalis* isolates of animal and human origin and PCR identification of genes within the VanA cluster. *Antimicrob. Agents Chemother.*, 408:1938-1940, 1996.

Alencar T.A., Mendonça E.C.L., Marques, V.F., Melo D.A., Rojas, A.C.M., Motta C.C.M., Santiago G.S., Dubenczuk F.C., Medeiros P.T.C., Coelho S.M.O. & Souza M.M.S. Aspectos das condições higiênico-sanitárias em unidades leiteiras em municípios do estado do Rio de Janeiro, Brasil e análise dos agentes bacterianos envolvidos na etiologia das mastites. *Rev. Bras. Med. Vet.*, 36:199-208, 2014.

Beninati C., Reich F., Muscolino D., Giarratana F., Panebianco A., Klein G. & Atanassova V. ESBL-Producing Bacteria and MRSA Isolated from Poultry and Turkey Products Imported from Italy. *Czech J. Food Sci.*, 33: 97–102, 2015.

Brazil. Ministry of Agriculture (MAPA). Order nº 31, 29th January 2002. Banning the use of arsenical and antimonial products. Federative Republic of Brazil, Brasilia, Section 1.

Brazil. Ministry of Agriculture (MAPA). Normative Ruling nº 09, 27th June 2003. Banishment of Chloramphenicol and nitrofurans. Federative Republic of Brazil, Brasilia, Section 1.

Brazil. Ministry of Agriculture (MAPA). Normative Ruling nº 09, 9th July 2009. Banishment of amphenicols, tetracyclines, beta-lactams (benzyl-penicillins and cephalosporins), quinolones, and systemic sulfonamides. Federative Republic of Brazil, Brasilia, Section 1.

Brazil. Ministry of Agriculture (MAPA). Normative Ruling nº 14, 17th July 2012. Banishment of spiramycin and erythromycin. Federative Republic of Brazil, Brasilia, Section 1.

Brazilian Poultry Association (UBABEF). Annual Report UBABEF 2010/2011, 2011. Disponible at: <http://www.brazilianchicken.com.br/files/publicacoes/ d220421fe22b294a31a584138b5fda95.pdf >. Accessed on: Fev 04, 2017.

Brazilian Poultry Association. (UBABEF). Annual Report Ubabef 2011/2012, 2012. Disponible at: <http://www.abef.com.br/ubabef/exibenoticiasubabef.php? notcodigo=2761>. Accessed on: Fev 04, 2017.

Cervantes H.M. The future of antibiotic growth promoters in poultry production. In: World´s Poultry Congress, XXIV, 2012, Salvador, Brazil, 16p. Disponible at: <http://www.facta.org.br/wpc2012-cd/pdfs/plenary/ Hector_M._Cervantes. pdf. Accessed on: Fev 04, 2017.

Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disk and Diluition Susceptibility Tests for Bacteria Isolated From Animals; Approved Standards; Second Informational Supplement, VET01-S2. *Clinical and Laboratory Standards Institute*, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2013.

Coelho S.M.O., Reinoso E., Pereira I.A., Soares L.C., Demo M., Bogni C. & Souza M.M.S Virulence factors and antimicrobial resistance of *Staphylococcus aureus* isolated from bovine mastitis in Rio de Janeiro. *Pesq. Vet. Bras.*, 29:369–374, 2009.

Coelho S.M.O., Pereira I.A., Soares L.C., Pribul B.R. & Souza M.M.S. Profile of virulence factors of *S. aureus* isolated from subclinical bovine mastitis in the state of Rio de Janeiro, Brazil. *J. Dairy Sci.*, 94:3305-3310, 2011.

Cucarella C., Tormo M.A., Úbeda C., Trotonda M.P., Monzón M., Peris C., Amorena B., Lasa I. & Penadés J.R. Role of biofilm-associated protein Bap in the pathogenesis of bovine *S. aureus*. *Infect Immun.*, 72:2177-2185, 2004.

Dahmen S., Métayer V., Gay E., Madec J.-Y. & Haenni M. Characterization of extended-spectrum beta-lactamase (ESBL)-carrying plasmids and clones of Enterobacteriaceae causing cattle mastitis in France. *Vet. Microbiol.*, 162:793-799, 2013.

Dalólio, F.S., Moreira. J., Valadares L. R., Nunes P.B., Vaz D.P., Pereira H. J., Pires A. J. & Cruz P.J.R. Aditivos alternativos ao uso de antimicrobianos na alimentação de frangos de corte. *Revista Brasileira de Agropecuária Sustentável*, 5:86-94, 2015.

David P. The challenge of antimicrobial resistance. *Lancet: Gastroenterol Hepatol.*, 1:173, 2016.

Devriese L.A., Vancanneyt M. & Baele, M. *Staphylococcus pseudintermedius* sp. nov., a coagulase positive species from animals. *Int. J. Syst. Evironment. Microbiol.*, 55:1569-1573, 2005.

Dias, G.E.A., Carvalho, B.O., Gomes, A.V.C., Medeiros, P.T.C., Sousa, F.D.R., Souza, M.M.S. & Lima, C.A.R. Oregano (*Origanum*

Rev. Bras. Med. Vet., 38(Supl.3):68-74, dezembro 2016

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vulgare L.) essential oil in the diet of broilers as balancing the intestinal microbiota. *Rev. Bras. Med. Vet.*, 37:108-114, 2015.

European Centre for Disease Prevention and Control. *Expert opinion on whole genome sequencing for public health surveillance.* Stockholm: ECDC; 2016.

European Medicines Agency. *Sales of Veterinary Antimicrobial Agents in 25 EU/ EEA Countries in 2011.* Third ESVAC Report (Eur. Med. Agency, London), 2013. Disponible at : http://www.ema.europa.eu/docs/ en_GB/document_library/ Report/2013/10/WC500152311.pdf. Accessed on: Ago 20, 2016.

Gouvêa R., Santos F.F., Aquino M.H.C. & Pereira V.L. Fluoroquinolones in Industrial Poultry Production, Bacterial Resistance and Food Residues: a Review. *Braz. J. Poult. Sci.*, 17:1-10, 2015.

Howard S.J., Hopwood S. & Davies S.C. Antimicrobial Resistance: A Global Challenge. S*ci. Translat. Med.*, 6:236ed10., 2014.

Howard P., Pulcini C., Levy Hara G., West R.M., Gould I.M., Harbarth S., Nathwani D, ESCMID Study Group for Antimicrobial Policies (ESGAP) & ISC Group on Antimicrobial Stewardship. An international cross-sectional survey of antimicrobial stewardship programmes in hospitals. *J. Antimicrob. Chemother.*, 70:1245 –1255, 2015.

Kateregga J.N., Kantume R., Atuhaire C., Lubowa M.N. & Ndukui J.G. Phenotypic expression and prevalence of ESBL-producing Enterobacteriaceae in samples collected from patients in various wards of Mulago Hospital, Uganda. *BMC Pharmacol. Toxicol.*, 16:1-6, 2015.

Li Y., Li Q., Du Y., Jiang X., Tang J., Wang J., Li G. & Jiang Y. Prevalence of Plasmid-Mediated AmpC beta-Lactamases in a Chinese University Hospital from 2003 to 2005: First Report of CMY-2-Type AmpC beta-Lactamase Resistance in China. *J. Clin. Microbiol.*, 46: 1317–1321, 2008.

Liebana E., Carattoli A., Coque T.M., Hasman H., Magiorakos A., Mevius D., Peixe L., Poirel L., Schuepbach-Regula G., Torneke K., Torren-Edo J., Torres C. & Threlfall J. Public Health Risks of Enterobacterial Isolates Producing Extended-Spectrum β-Lactamases or AmpC β-Lactamases in Food and Food-Producing Animals: An EU Perspective of Epidemiology, Analytical Methods, Risk Factors, and Control Options. *Clin. Infect. Dis.*, 56:1030-7, 2013.

Locatelli C., Caronte I., Scaccabarozzi L., Migliavacca R., Pagani L. & Moroni P. Extended-spectrum beta-lactamase production in *E. coli* strains isolated from clinical bovine mastitis**.** *Vet. Res. Commun.*, 33:S141-S144, 2009.

Marques V.F., Motta C.C., Soares B.S., Melo D.A., Coelho S.M.O., Coelho I.C., Barbosa H.S. & Souza. M.M.S. Biofilm production and beta-lactamic resistance in *S. aureus* bovine mastitis Brazilian isolates. *Braz. J. Microbiol.*, 48:118-124, 2017

Melo D.A., Coelho I. S., Motta C.C., Rojas A.C.C.M., Dubenczuk F.C., Coelho S.M.O. & Souza M.M.S. Impairments of *mec*A gene detection in bovine *Staphylococcus* spp. *Braz. J. Microbiol.*, 45:1075-1082, 2014.

Mendonça E.C.L., Marques V.F., Melo D.A., Alencar T.A., Coelho I.S., Coelho S.M.O., Souza M.M.S. Caracterização fenogenotípica da resistência antimicrobiana em *Staphylococcus* spp. isolados de mastite bovina. *Pesq. Vet. Bras.*, 32:859-864, 2012.

Moon J.S., Lee A.R., Kang H.M., Lee E.S., Kim M.N., Paik Y.H., Park Y.H., Joo Y.S., Koo H.S. Phenotypic and genetic antibiogram of methicillin-resistant Staphylococci isolated from bovine mastitis in Korea. *J. Dairy Sci.*, 90:1176-1185, 2007.

Motta C.C., Rojas A.C.M., Dubenczuk F.C., Botelho L.A.B., Moreira B.M., Coelho S.M.O., Coelho I.S. & Souza M.M.S. Verification of molecular characterization of coagulase positive *Staphylococcus* from bovine mastitis with matrix-assisted laser desorption ionization, time-offlight mass spectrometry (MALDI-TOF MS) mass spectrometry. *Af. J. Microbiol. Res.*, 8:3861-3866, 2014.

Nääs I.A., Mollo Neto M., Canuto S.A.,Waker R., Oliveira D.R.M.S. & Vendrametto O. Brazilian chicken meat production chain: a 10-year overview. *Rev. Bras. Cienc. Avic.*, 17:87-94, 2015.

Park K.R., Park C.S. & Kim B.G. An enzyme complex increases in vitro dry matter digestibility of corn and wheat in pigs. *SpringerPlus*, 5:598, 2016.

Ramchandani M., Manges A.R., DebRoy C., Smith S.P., Johnson J.R. & Riley L.W. Possible animal origin of human-associated, multidrug-resistant, uropathogenic *Escherichia coli. Clin. Infect. Dis.*, 40: 251-257, 2005.

Raza A., Muhammad G., Sharif S., Atta A. Biofilm producing *S. aureus* and bovine mastitis: a review. *Mol. Microbiol. Res.*, 3:1–8, 2013.

Saei H.D. *coa* types and antimicrobial resistance profile of *S. aureus* isolates from cases of bovine mastitis. *Comp. Clin. Pathol.*, 21:301-307, 2012.

Santiago G.S., Lasagno M.C., Alencar T.A., Ribeiro L., Oliva M.S., Souza M.M.S & Coelho S.M.O. AmpC β-lactamase production in enterobacteria associated with bovine mastitis in Brazil. *Afr. J. Microbiol. Res.*, 9:503-508, 2015.

Soares L.C., Pereira I.A., Pribul B.R., Oliva M.S., Coelho S.M.O. & Souza M.M.S. Antimicrobial resistance and detection of *mec*A and *bla*Z genes in coagulase-negative Staphylococcus isolated from bovine mastitis. *Pesq. Vet. Bras.*, 32:692-696, 2012.

Soares B.S. *Aplicação de técnicas moleculares para o monitoramento da diversidade genética de Staphylococcus aureus em ambientes de produção leiteira.* 2017. 82p. Tese (Ciências Veterinárias) – Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, Seropédica. 2017.

Souza M.M.S., Coelho S.M.O., Pereira I.A., Soares L.C., Pribul B.R. & Coelho I.C. Antibiotic Resistance in *Staphylococcus* Species of Animal Origin, p.273-302. In: Pana M. (Ed.) *Antibiotic Resistant Bacteria - A Continuous Challenge in the New Millennium*, InTech, 2012. Disponible at: http://www.intechopen.com/books/ antibiotic-resistant-bacteria-a-continuous-challenge-in-the-newmillennium/antibiotic-resistance-in-staphylococcus-species-of-animal-origin. Accessed on: Fev 04, 2017.

Wells C.L., Henry-Stanley M.J., Barnes A.M.T., Dunny G.M., Hess D.J. Relation between antibiotic susceptibility and ultrastructure of *Staphylococcus aureus* biofilms on surgical suture. *Surg. Infect.*, 12:297-305, 2011.