

Classification of antimicrobial resistance using artificial neural networks and the relationship of 38 genes associated with the virulence of *Escherichia coli* isolates from broilers¹

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ABSTRACT. Rocha D.T., Salle F.O., Perdoncini G., Rocha S.L.S., Fortes F.B.B., Moraes H.L.S., Nascimento V.P. & Salle C.T.P. 2015. **Classification of antimicrobial resistance using artificial neural networks and the relationship of 38 genes associated with the virulence of *Escherichia coli* isolates from broilers.** *Pesquisa Veterinária Brasileira* 35(2):137-140. Centro de Diagnóstico e Pesquisa em Patologia Aviária, Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 8824, Porto Alegre, RS 91540-000, Brazil. E-mail: cdpa@ufrgs.br

Avian pathogenic *Escherichia coli* (APEC) is responsible for various pathological processes in birds and is considered as one of the principal causes of morbidity and mortality, associated with economic losses to the poultry industry. The objective of this study was to demonstrate that it is possible to predict antimicrobial resistance of 256 samples (APEC) using 38 different genes responsible for virulence factors, through a computer program of artificial neural networks (ANNs). A second target was to find the relationship between (PI) pathogenicity index and resistance to 14 antibiotics by statistical analysis. The results showed that the RNAs were able to make the correct classification of the behavior of APEC samples with a range from 74.22 to 98.44%, and make it possible to predict antimicrobial resistance. The statistical analysis to assess the relationship between the pathogenic index (PI) and resistance against 14 antibiotics showed that these variables are independent, i.e. peaks in PI can happen without changing the antimicrobial resistance, or the opposite, changing the antimicrobial resistance without a change in PI.

INDEX TERMS: *Escherichia coli*, artificial neural networks, antimicrobials agents, broilers.

RESUMO. [Utilização de redes neurais artificiais para a classificação da resistência a antimicrobianos e sua relação com a presença de 38 genes associados a virulência isolados de amostras de *Escherichia coli* provenientes de frangos de corte.] *Escherichia coli* patogênica (APEC) para as aves é responsável por vários processos patológicos em aves, sendo considerado como uma das principais causas de morbidade e mortalidade, associado com perdas econômicas para a indústria avícola. O objetivo do presente trabalho foi demonstrar que é possível prever a resistência antimicrobiana de 256 amostras de APEC uti-

lizando 38 genes responsáveis por distintos fatores de virulência, através de um programa computacional de redes neurais artificiais (RNAs). O segundo objetivo foi verificar por análise estatística a relação entre o índice de patogenicidade (IP) e a resistência aos 14 antimicrobianos. Os resultados demonstraram que as RNAs foram capazes de realizar a classificação correta do comportamento das amostras APEC com uma amplitude de 74,22 a 98,44%, desta forma tornando possível prever a resistência antimicrobiana. A análise estatística realizada para verificar a relação entre o IP e a resistência aos antimicrobianos demonstrou que estas variáveis são independentes, ou seja, podem haver picos no IP sem alteração na resistência, ou até mesmo o contrário, alteração na resistência antimicrobiana sem mudança no IP.

TERMOS DE INDEXAÇÃO: *Escherichia coli*, redes neurais artificiais, agentes antimicrobianos, frangos.

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INTRODUCTION

APEC belongs to the Enterobacteriaceae family. It is responsible for various pathological processes in poultry, acting as a primary or secondary agent causing lesions such as: aerocarditis, pericarditis, perihepatitis, peritonitis, salpingitis, omphalitis, cellulitis, among others (Kabir 2010).

The resistance to antimicrobial drugs is a major problem in many pathogens. The extensive use of antimicrobials in medicine, veterinary medicine and agriculture provides highly selective conditions to spread R plasmids. The R plasmids and other sources of resistance genes represent a significant limitation to use of antimicrobial as a chemotherapeutic agent. The rates of resistance of bacteria against the tested antibiotics are increasing, representing a serious global problem. *Escherichia coli* strains isolated from poultry are frequently resistant to more than one drug (Zanata et al. 2004, Madigan et al. 2010). Several studies have reported the issue of antimicrobial resistance by APEC.

Analyzing 100 strains of *E. coli* of avian origin, the results showed that the antimicrobial resistance of the samples was 23% for enrofloxacin, 26% for cephalexin (less resistant) and 100% for erythromycin, tetracycline to 99% (more resistant) (Zakeri & Kashefi 2012).

In another research, 251 APEC samples were analyzed. The results were: 102 (40.6%) and 67 (26.7%) were resistant to tetracycline and ampicillin, respectively. It was observed resistance to trimethoprim-sulfamethoxazole (12.4%), streptomycin (10.8%), spectinomycin (9.6%), neomycin (6.0%) and florfenicol (2.0%), but no resistance was observed to gentamicin, ciprofloxacin and ceftiofur (Obeng et al. 2012).

E. coli strains have shown resistance to at least two classes of antimicrobials. Both in human medicine and in veterinary medicine, this fact has caused a growing impact on viable therapeutic options. It was also considered one of the greatest health problems according to the (WHO) World Health Organization (Baum & Marre 2005, Pons et al. 2012). Consequently, it was observed the necessity of adopting new methodologies capable of classifying antimicrobial resistance. It is believed that one of the tools which has potential to help in this task is the utilization of ANNs, for decision making.

The term (AI) artificial intelligence first occurred in 1956 at a meeting with scientists from various fields of knowledge, interested in creating intelligent machines. AI is a way to simulate the natural intelligence, inspired by the structure and function of biological neurons (Artero 2009).

Artificial neural networks consist of a method to solve problems and, unlike conventional systems, they do not perform logical operations, but discover the relationships or rules of data and examples and test all possibilities in parallel. The neural network is based on data which are available to extract a general model. To make it possible, the system must be supplied with actual data that allows its training and learning (Salle et al. 2009).

The main aim of this study was to demonstrate that it is possible to predict antimicrobial resistance of 256 samples (APEC) using 38 different genes responsible for virulence

factors, through a computer program of artificial neural networks (ANNs). A second target was to find by statistical analysis the relationship between (PI) pathogenicity index and resistance to 14 antibiotics.

MATERIALS AND METHODS

The data used in this study was collected in a database generated at the Center of Diagnosis and Research in Avian Pathology, Veterinary Faculty, Federal University of Rio Grande do Sul - Brazil. Information on 246 isolates of *Escherichia coli* were used as follows:

Characterization of 38 genes associated with pathogenicity (*crl*, *ireA*, *cnf1/2*, *tia*, *sat*, *fyuA*, *mat*, *sfa/focCD*, *malX*, *afa/draB*, *neuC*, *iha*, *hrlA*, *fimC*, *pic*, *hlyA*, *kpsMTII*, *sitDep*, *ompA*, *iroN1*, *gimB*, *sitD*, *tratT*, *ibeA*, *chuA*, *vat*, *tsh*, *iucD*, *cvi/cva*, *papC*, *irp2*, *iss*, *EAST1*, *felA*, *iutA*, *cvaC*, *papG*, *fimH*);

Antimicrobial resistance to 14 antibiotics (amikacin, amoxicillin/clavulanic acid, ampicillin, cephalexin, ceftiofur, cefuroxime, ciprofloxacin, clindamycin, cotrimoxazole, enrofloxacin, gentamicin, norfloxacin, ofloxacin and tetracycline);

The artificial neural networks were constructed using the Neuroshell Classifier 2.1 software (Ward Systems Group, Inc., Frederick, MD, USA, 1997-2000).

The available results were transformed into a binary form. Thus, the negative results were represented by number 0 and the positive ones by 1. This criterion was used for sensitivity (0) or resistance (1) to the 14 antibiotics, to the absence (0) or presence (1) of genes associated with pathogenicity.

The neural network used was backpropagation kind. It usually has at least 3 layers of neurons, each of which is "connected" to the neurons in the next layer. These connects are weights which are applied to values passed from one neuron to the next. Input values in the first layer are weighted and passed to the second (hidden) layer. Neurons in the hidden layer produce outputs that are based upon the sum of weighted values passed to them. The hidden layer passes values to the output layer in the same fashion, and the output layer produces the desired results.

The network "learns" by adjusting the interconnection weights. The answers which the network produces are repeatedly compared with the correct ones, and each time the connecting weights are slightly adjusted in the direction of the correct answers (Artero 2009).

In this research, the word "input" is used to identify the available or chosen variables, which were used to calculate the predictive model. The term "output" means the variables to be predicted (Salle 2001).

Based on the methodology used by Salle (2009), the inputs to construct the artificial neural networks were the 38 genes associated with pathogenicity. The outputs were the behavior of APEC strains against 14 antibiotics.

Statistical analysis was performed with the aid of JMP® 9.0.1 software (SAS Institute Inc., 2010) to observe the significant difference between the mean levels of pathogenicity and the sensitivity and resistant to the 14 antibiotics.

RESULTS

Table 1 shows the characteristics of artificial neural networks constructed with 38 genes associated with virulence. The outputs were the resistance or sensitivity to 14 antibiotics. The correct classifications were between 74.2 and 98.44%. A high specificity and sensitivity were observed as well (Salle 2009).

Table 1. Classification of bacterial resistance (intermediate, resistant or sensitive) of 256 samples *Escherichia coli* to 14 antibiotics using artificial neural networks, as input the 38 genes associated with virulence

Outputs	Inputs	Correct classifications (%)	S (i) ^a	E (i) ^b	S (r) ^c	E (r) ^d	S (s) ^e	E (s) ^f
Amikacin	Genes	97,27	0,0	1,0	0,0	1,0	1,0	0,0
Amoxicillin + Clavulanic	Genes	98,05	1,0	1,0	0,0	1,0	1,0	0,37
Ampicillin	Genes	82,42	0,72	1,0	0,36	0,98	0,98	0,41
Vet 1854_	Genes	94,92	0,6	1,0	0,41	0,99	0,99	0,5
Cephalexin								
Ceftiofur	Genes	96,88	0,57	1,0	0,71	1,0	1,0	0,61
Cefuroxime	Genes	96,09	0,0	1,0	0,28	1,0	1,0	0,16
Ciprofloxacin	Genes	85,94	0,75	1,0	0,55	0,97	0,97	0,61
Clindamycin	Genes	98,44	0,5	1,0	1,0	0,5	0,5	1,0
Cotrimoxazole	Genes	74,22	1,0	1,0	0,72	0,75	0,74	0,73
Enrofloxacin	Genes	75,78	0,42	0,98	0,53	0,94	0,94	0,55
Gentamicin	Genes	88,67	-	-	0,35	0,99	0,99	0,35
Norfloxacin	Genes	88,28	0,55	1,0	0,34	1,0	1,0	0,42
Ofloxacin	Genes	89,45	1,0	1,0	0,24	0,99	0,99	0,5
Tetracycline	Genes	77,73	1,0	1,0	0,87	0,63	0,62	0,87

^a S (i) = sensitivity for isolates with intermediate resistance; ^b E (i) = specificity for isolates with intermediate resistance; ^c S (r) = sensitivity to resistant isolates; ^d E (r) = specificity for resistant isolates; ^e S (s) = sensitivity for sensitive isolates; ^f E (s) = specificity for sensitive isolates.

The correct classification was above 80% for most antimicrobials. Sensitivities of the amplitude were rated from 0% to 100% for the intermediate samples, from 0% to 87% for the resistant samples and from 62% to 100% for the sensitive samples. The specificities, were from 98% to 100% for the intermediate samples, from 50% to 99% for resistant and from 0% to 87% for the sensitive ones.

The results obtained from statistical analysis of the pathogenicity index and the 14 antimicrobials that were analyzed showed no significant differences between the averages.

DISCUSSION

This study discussed the antimicrobial resistance of APEC in an innovative way, using artificial neural networks (ANN) as a tool for decision making methodology inserted in the line of research of the Center of Diagnosis and Research in Avian Pathology, (mathematical models) in poultry.

ANNs have been used in various fields including agriculture, bioinformatics, robotics, health, finance and others. This fact is proved in numerous articles published using this tool.

In a work by Salle et al. (2001), the researchers studied the use of artificial neural networks to predict the performance parameters in breeding birds. Data from 11 broiler breeder flocks was recorded between November 11th, 1997 and October 1st, 1999. The method allows decision making by the technical staff, based on objective criteria, scientifically obtained.

Salle et al. (2011), built artificial neural networks using the following data inputs: the pathogenicity index of the samples, lesions provoked by the inoculation in day-old-chicks, characterization of 7 genes associated with pathogenicity, the biochemical behavior (adonitol, arginine,

dulcitol, ornithine, raffinose, salicin, sucrose), motility and the origin of the samples. The outputs data used were the resistance or sensibility to 14 antimicrobials obtained by Salle (2009). In that study artificial neural networks were able to perform the correct classification of antimicrobial behavior of the samples with an amplitude of 90.24 to 100%, demonstrating one more time the affectivity of this tool in this specific research line allowing the development of future studies.

According to Rocha (2013) it was possible, in a simplified way, using only the 38 genes associated with virulence, to obtain conclusive results regarding antimicrobial resistance.

Drawing a parallel between Salle et al. (2011) and the present study, it was observed that the performed work did not need additional information from the sample to conclude the network. It is noted that many times the samples received at the laboratory, did not contain additional information which, until then, would be crucial for making the network.

The information received about virulence genes found in a APEC sample becomes a valuable result to the veterinarian who is working in the field, because using this information (data) along with ANNs software it is possible to predict the behavior of this sample against antimicrobial agents, thus showing an alternative to antimicrobial susceptibility testing.

CONCLUSIONS

This study demonstrates that it is possible to predict resistance and antimicrobial sensitivity using as inputs only 38 genes associated with pathogenicity obtained through PCR protocols developed in the laboratory.

It also concluded that: in the presence of infection by *Escherichia coli*, one can now use technologies like polymerase chain reaction (PCR), combined with the use of artificial neural networks to define the best treatment for that sample. Thus, treatment against this pathogen becomes more agile and precise.

The results from statistical analysis of the pathogenicity index and the 14 antimicrobials which were analyzed in this study showed no significant differences between the averages.

It was also noticed that these variables were independent, namely, it is possible to observe peaks of PI without any changes in antimicrobial resistance or the opposite changing the antimicrobial resistance without a change in the PI.

REFERENCES

- Artero A.O. 2009. Inteligência artificial teórica e prática. Vol.1. Livraria da Física, São Paulo. 230p.
- Baum V.H. & Marre R. 2005. Antimicrobial resistance of *Escherichia coli* and therapeutic implications. Int. J. Med. Microbiol. 295:503-511.
- Kabir S.M.L. 2010. Avian colibacillosis and salmonellosis: a closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. Int. Environ. Res. Health 7:89-114.
- Madigan M.T., Martinko J.M., Dunlap P.V. & Clark D.P. 2010. Resistência a fármacos antimicrobianos e a descoberta de fármacos, p.802-808. In: Ibid. (Eds), Microbiologia de Brock. 12^a ed. Artmed, Porto Alegre.
- Obeng A.S., Rickard H., Ndi O., Sexton M. & Barton M. 2012. Antibiotic resistance, phylogenetic grouping and virulence potential of *Escherichia*

- coli* isolated from the faeces of intensively farmed and free range poultry. Vet. Microbiol. 154:305-315.
- Pons M.J., Mosquito S., Ochoa T.J., Vargas M., Molina M., Lluque A., Gil A.I., Ecker L., Barbaletta F., Lanata C.F., Valle L.J.D. & Ruiz J. 2012. Niveles de resistencia a antimicrobianos, en especial a quinolonas, en cepas de *Escherichia coli* comensales en niños de la zona periurbana de Lima, Perú. Revta Peru. Med. Exp. Salud Publica 29:82-86.
- Rocha D.T. 2013. Utilização de redes neurais artificiais para a classificação da resistência a antimicrobianos e sua relação com a presença de 38 genes associados a virulencia isolados de amostras de *Escherichia coli* provenientes de frangos de corte. Dissertação de Mestrado em Ciências Veterinárias, Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS. 49p.
- Salle C.T.P., Guahyba A.S., Wald V.B., Silva A.B., Salle F.O. & Fallavena L.C.B. 2001. Use of artificial neural networks to estimate production parameters of broiler breeders in the breeding phase. Revta Bras. Ciênc. Avícola 3:257-264
- Salle F.O. 2009. Utilização de inteligência artificial (redes neurais artificiais) para a classificação da resistência a antimicrobianos e do comportamento bioquímico de amostras de *Escherichia coli* isoladas de frangos de corte. Tese de Doutorado em Ciências Veterinárias, Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS. 88p.
- Salle F.O., Fortes F.B.B., Rocha A.C.G.P., Rocha S.L.S., Souza G.F., Moraes H.L.S., Nascimento V.P. & Salle C.T.P. 2011. Use of artificial intelligence (artificial neural networks) to classify antimicrobial resistance from *Escherichia coli* samples isolated from broilers. Anais 60th Western Poultry Disease Conference, Sacramento, CA, p.127. (Resumo)
- Zakeri A. & Kashefi P. 2012. Antimicrobial susceptibilities of avian *Escherichia coli* isolates in Tabriz, Iran. African J. Biotechnol. 11:4467-4470.
- Zanata G.F., Kanashiro A.M.I., Castro A.G.M., Cardoso A.L.S.P., Tessari E.N.C. & Pulici S.C.P. 2004. Susceptibilidade de amostras de *Escherichia coli* de origem aviária a antimicrobianos. Arqs Inst. Biológico, São Paulo, 71:283-286.