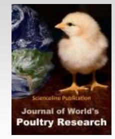
JWPR

Journal of World's Poultry Research

2019, Scienceline Publication

J. World Poult. Res. 9(2): 89-97, June 25, 2019

Review Paper, PII: S2322455X1900012-9



A Meta-Analysis on Antibiotic Residues in Meat of Broiler Chickens in Developing Countries

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Received: 02 May 2019

Accepted: 01 June 2019

ABSTRACT

Present study consisted of performing a meta-analysis on data about the detection of antibiotic residues in chicken meat achieved from all over the researches with a wide collection and very strict selection criteria of data. The databases were searched quantitative inputs from the available scientific publications using important keywords, in order to evaluate all studies about antibiotic residue and detection methods and the reliability of the results obtained by the international researchers. Then an advanced statistical analysis on collected data was done, the first phase was a descriptive study of positive and negative cases followed by a modeling of two cases with a prediction of the values obtained and ended with an analysis of the main compounds (population size, residue detection methods and positive case rates). All performed steps are reported in detail. The results indicated that the accuracy of the detection technique is a factor that influenced on reports of residues, and caused differences in reports, there are still antibiotic residues in meat of intensively broiler chicken farms (45. 26% of the samples analysed are positive), It is concluded that residue detection requires a high-precision qualitative analysis protocol.

Key words: Antibiotic residues, Broiler chicken, Databases, Meta-analysis.

INTRODUCTION

Antibiotic residues in food of animal origin remain a topical issue throughout the world, because the massive use of these molecules, for preventive and curative therapeutic purposes and also as food additives or growth promoters. (Preziosi et al., 2012; Berghiche et al., 2018). The lack of international regulations to control the use of antibiotics in poultry farming, especially in developing countries (possession, distribution and use are delayed), where many farmers treat their animals without knowing the conditions and quantities to administer or the withdrawal times (Mills et al., 2015).

Recent literature suggested that the term meta-analysis refers precisely to Glass (1976), who refers to the fact that results are evaluated at a higher level and from a more general perspective than the original studies. Glass thus focuses on the analysis of analyses. This method of analysis appeared in the 1970s in the fields of classical experimental sciences and psychotherapy (Webster, 2019).

From the 1990s onwards, the first meta-analyses appeared in the field of economics, particularly in environmental economics (Boyle et al., 1994; Schwartz, 1994; Smith and Huang, 1995) and also in labour economics (Jarrell and Stanley, 1990; Hedges et al., 1994; Card and Krueger, 1995).

Meta-analysis is a statistical approach, which aims to combine the results of a series of available and independent studies on the subject and then make a reproducible synthesis (Bouras et al., 2019).

The principles of meta-analysis are as follows: It should include all available trials for the treatment studied, positive and negative, published and unpublished (exhaustive research) (Marshall et al., 2019). It must be based on a precise protocol, to avoid any bias in the choice of studies included. Finally, this statistical method is applied to contextualize the probability of the outcome of all studies (Cervesato et al., 2019).

In total there are 23 studies that match the criteria for inclusion in this meta-analysis. In detail there were 17 studies in eight African countries (Congo, Sudan,

To cite this paper: Berghiche A, Khenenou T and Labiad I (2019). A Meta-Analysis on Antibiotic Residues in Meat of Broiler Chickens in Developing Countries. *J. World Poult. Res.*, 9 (2): 89-97. http://jwpr.science-line.com/

Morocco, Nigeria, Senegal, Tunisia, Egypt and Algeria), four studies of them in three Asiatic countries (Turkey, Iraq and Palestine) and one in one European country (Bulgaria).

The uncontrolled use of antibiotics to form high-risk residues for the consumer, this work is focused on these residues through studies performed for its detection by the different methods based on a statistical analysis of the meta data available on this subject, the objective of present research was to evaluate the relationship between antibiotic therapy in broiler chickens and residues of antibiotic compounds in the white meat, a statistical analysis was based of the meta-data available on the subject.

The meta-analysis, which makes it possible to estimate this correlation by synthesizing similar studies that have common characteristics.

MATERIALS AND METHODS

Experimental design

The protocol was developed prior to the meta-analysis. It is defined in three steps described below: Identification, obtaining and selection of articles that were related to present research criteria, data extraction and statistical analysis.

Defining the variables

Different factors are likely to influence the presence of antibiotic residues in each study that included:

- The organic materials used broiler chicken meat or offal (liver, gizzard).
- Type of food (feed additive).
- Antibiotic treatments performed.
- The route and dose of administration.
- Breeding method (traditional or modern).
- Breeding condition, include the type of soil used, relative humidity, the total number of animals per farm, the surrounding temperature and the level of hygiene are all environmental factors that can vary from one farm to another.
- The methods used for the study are sources of variability.
- The age and weight of the chickens studied may also be different.
- Judgement criteria, included the endpoint was the variable measuring at the end of the experiment to define the effectiveness of the test product.

In order to understand all the factors of variation in order to define a protocol that avoids sources of bias as much as possible.

Searching for articles related to the issue

After defining the main characteristics of the studies, it is necessary to search them exhaustively.

Pubmed. Pubmed being a free, easy-to-use and very complete search database, firstly to perform present search using this site.

The search performed is as follows:

Reading of bibliographies. After sorting the articles obtained through pubmed, and reading the bibliography of each article, in order to retrieve other articles.

Google scholar. Google scholar, allowed the searching of academic work. It was used last to ensure that no studies dealing had been forgotten.

Selection of usable articles. Initially, articles were selected based on their title and abstract, when available. The rest of the selection was made after obtaining the entire items; the selected items should have certain characteristics, which are sample size, type of antibiotic sought and method used for detection.

Selection on title and summary. The first selection was made simply from the title of the articles, also keywords related to antibiotic residues in broiler chicken meat. The selected articles were further developed by reading their abstracts. A decision was taken to include only articles in English or French languages. This first phase excluded non-native language articles and studies on the detection of antibiotic residues in foodstuffs apart on white meat. Unfortunately, the reviewing of titles and abstracts sometimes leaves some doubt as to whether or not a study can be included in the meta-analysis. In this case the selection was made base on the entire article.

Selection on the entire article. The quality of some articles was not always adequate and some articles just did not meet the requirements of the meta-analysis.

The authors of this study decided that evaluate the quality of the article for including in present study base on following condition:

- Animal species has been specified, is the broiler chicken.
- The antibiotics used in poultry farming that anybody looking for them (like Tetracycline, Sulfonamides, Aminosides, Macrolide, Beta lactamine, Tylosin, Colistin etc.).
- The research method used (four boxes reference method)

Characteristics of the articles. The details of the articles, those were not essential, but interesting and allowing to explain some calculations. The exclusion criteria for each item were clearly stated, in this study only those concerning the detection of chloramphenicol was excluded, because this molecule is currently not commercialized (Fowler, 1992).

Studies included in the meta-analysis

The contamination of animal food origin by antibiotic residues has been reported by many authors, the selected research data are listed in the following summary table (Table 1).

Table 1. Summary table of studies concerned by the meta-analyses on antibiotic residues in meat of broiler chickens (Data included)

Study	Authors	Years	Counrty
01	Okombe et al.	2016	Congo
02	Hind et al.	2014	Sudan
03	Titouche et al.	2016	Algeria
04	Karmi	2014	Egypt
05	Mansouri	2007	Algeria
06	Omotoso	2015	Nigeria
07	Benghalem et al.	2016	Algeria
08	Alambedji et al.	2004	Senegal
09	Rezgui	2009	Tunisia
10	Abiola	2005	Senegal
11	N'kaya	2004	Senegal
12	Ramdane	2015	Algeria
13	Ben Mohand	2008	Algeria
14	Chaiba et al.	2017	Morocco
15	Ezenduka, et al.	2014	Nigeria
16	Onurdağ et al.	2013	Turkey
17	Shareef et al.	2009	Iraq
18	Elmanama and Albayoumi.	2016	Palestine
19	Shamsa.	2013	Iraq
20	Al Pavlov et al.	2008	Bulgaria
21	Berghiche et al.	2017	Algeria
22	Berghiche et al.	2018	Algeria

Studies not included in the meta-analysis

The objective of this study was to detect antibiotic (chloramphenicol) residues in chicken liver, kidney and muscle by three methods: four box methods, high performance liquid chromatography and enzyme-linked immunosorbent assay test (Tajik et al., 2010), this study was excluded because the use of the antibiotic concerned is currently banned so the determination of its minimum residual limit is irrelevant. Chloramphenicol is a broad-spectrum antibiotic active against Gram-positive and Gram-negative bacteria. It is an effective therapeutic agent for the treatment of many animal infections. However, historical epidemiological data have shown that its use in humans may be associated with hematological disorders, including aplastic anemias. During the evaluation, it was not possible to set a threshold value base on the available data. This inability to set the threshold value and the shortcomings of the documents have led to a classification as a prohibited substance for use in food-producing animals in the European community since 1994 (Mensah, 2014).

Statistical analysis

The collected data are logged and processed using the program (Microsoft Office Excel, 2007) to perform the description and evaluation, for the advanced statistical part a various statistical software was used (XLSTAT, Past 3 and Pro Origin).

The first phase was a descriptive study of positive and negative cases followed by a modelling of two cases with a prediction of the values obtained and ended with an analysis of the main compounds (population size, residue detection methods and positive case rates), a value of (P=0.05) which is considered significant throughout all the results obtained

RESULTS

Summary and description of the studies concerned by the meta-analysis

In a summary table an exhaustive description of the studies already carried out in the field of detection of antibiotic residues in the chicken meat in an exhaustive way (Table 2).

Prevalence of antibiotic residues across the different studies

As shown in figure 1, the presence of antibiotic residues with a rate of 45.30% was detected in 1144 cases out of a total of 2525. There is a significant difference (P<0.05) between the study areas with the highest rate of antibiotic residues reported in Africa with 649 positive cases followed by Europe and Asia with 462.460 positive cases respectively (Nisha, 2008; Berghiche, 2019). A boxplot is a graph that gives a good indication of how the values of the data are distributed, the arrangement of the data on its variability or dispersion.

The comparison between the two distributions shows that the different results that are negative are compact (Figure 2A) and for the positive ones are dispersed (Figure 2B).

Table 2. Recuperative table of results obtained across the different studies

No.	Country	Total No. of analysed samples	Cas positives	Methods used	Antibiotics researched
01	Congo	67	7 (10.44%)	Four boxes	Tetracycline (100%) Tylosine (100%) Colistine (75%)
02	Soudan	221	60 (27%)	Three plates	Quantitative detection
03	Algeria	145	124 (85.51%)	Four boxes	Betalactamine /tetracycline (75.81%) Macrolide /betalactamine (44.35%) Sulfamide (36.29%) Aminoside (13.71%)
04	Egypt	50	42 (83.33%)	Four boxes	Beta lactamine (0%) Tetracycline (51%) Sulfamide (53%) Macrolide (0%) Aminoside (15%) Quinolone (39%)
05	Algeria	120	79 (65.7%)	Four boxes	Sulfamide (87.65%) Macrolide /tetracycline (43.20%) Betalactamine /Tétracycline (12.34%) Aminosides (11.11%)
06	Nigeria	80	44 (55%)	one box	Ciprofloxacin (51%) Norfloxacin (55%) Ofloxacin (40%)
07	Algeria	50	32 (64%)	Four boxes	Betalactamine /Tétracycline Bétalactamine /macrolide Sulfamide Aminoglucoside
08	Senegal	37	21 (50.5%)	Four boxes	Tetacycline (14%) Sulfamide (8%) Nitrofuraines (41%) Chloramphenicol (5%)
09	Tunisia	12	2 (16.66%)	Recepteur assay	Tétracycline Sulfamide Quinolone
10	Senegal	100	20 (20%)	Four boxes	Quantitative detection
11	Senegal	91	9 (10%)	Four boxes -thin-layer chromatography-Recepteur assay	Aminosides (8.33%%) Sulfamide (14.28%) Beta lactamine - Tetracyclines (17.86%) Beta lactamine – Macrolides (57.14%)
12	Algeria	90	54 (60%)	Four boxes	Betalactamine /tetracycline (37.5%) Sulfamide (18.75%) Aminoside (12.5%) Beta lactamine /macrolide(0%)
13	Algeria	30	16 (53.33%)	Four boxes/ high performance liquid chromatography	Beta lactamine (peni G) (100%) Macrolide (6.66%)
14	Morocco	50	18 (36.15 %)	High-performance liquid chromatography coupled to diode array UV detection and mass spectrometry / Four boxes	Tetracycline (26%) Sulfonamides (6%) Aminosides (6%) Macrolide / Beta lactamine (12%)
15	Nigeria	70	42 (60%)	Thin-layer chromatography	Quantitative detection
16	Turkey	127	58 (45.7%)	Enzyme-linked immunosorbent assay	Quinolone (45.7%)
17	Iraq	75	39 (52%)	Thin-layer chromatography	Oxytetracycline (24%) Sulfamide (24%) Neomycin (0%) Gentamycin (0%)
18	Palestine	365	88 (24.1%)	Four boxes	Tetracycline (43.15%) Aminoside (27.36%) Betalactamine (21%) Macrolide (8.42%)
19	Iraq	40	17 (42.5%)	Thin-layer chromatography	Sulfamide (27.5%) Oxytetracycline (20%) Neomycin (10%)
20	Bulgaria	462	35 (15.8%)	Four boxes	Quantitative detection
21	Algeria	50	18 (34%)	Four boxes	Tetracycline Erythromycin
22	Algeria	120	58 (69.6%)	Four boxes / Spectrophotometry	Quantitative detection
Total		2695	1220 (45.26%)		/

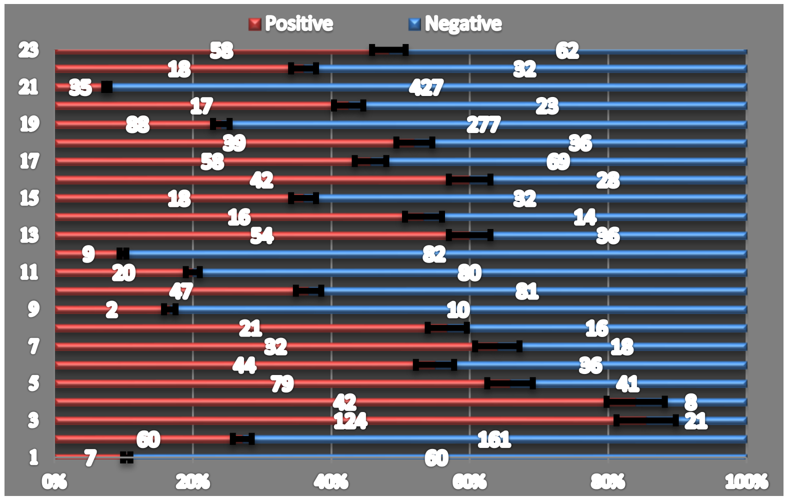

Figure 1. Recuperative results obtained of the detection of antibiotic residues used in poultry farming across the different studies in developing countries.

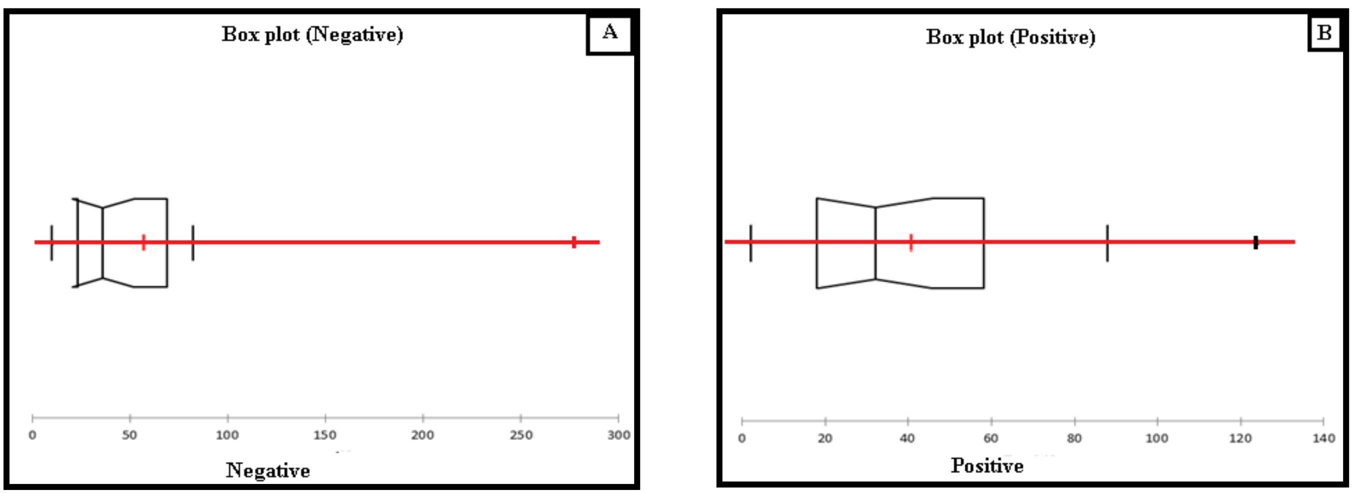

Figure 2. Results obtained from the detection of antibiotics used in poultry farming presented in Box Plot: (A) Positive cases, (B) Negative cases

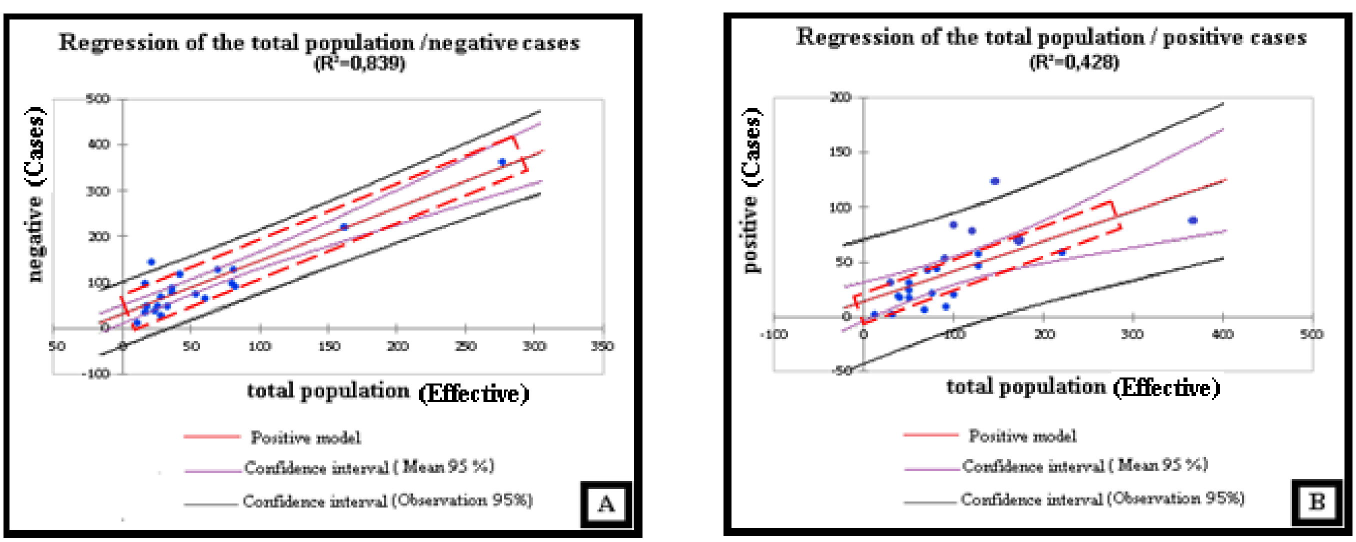

Figure 3. Modelisation of the results obtained from the detection of antibiotics used in poultry farming by linear regression in developing countries: (A) Negative cases**,** **(**B) Positive cases (R^2: correlation coefficient)

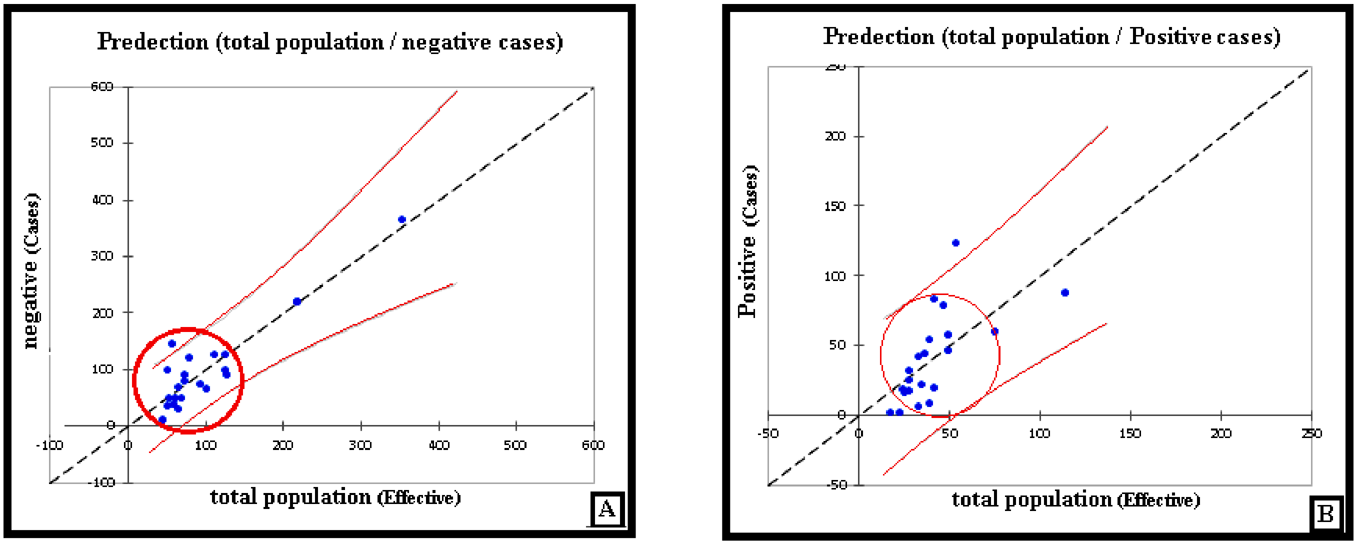

Figure 4. Results obtained from the detection of antibiotics used in poultry farming in developing countries (predetermination of the density of the values): (A) Positive cases**, (**B) Negative cases (Specified section: aggregation of prediction values)

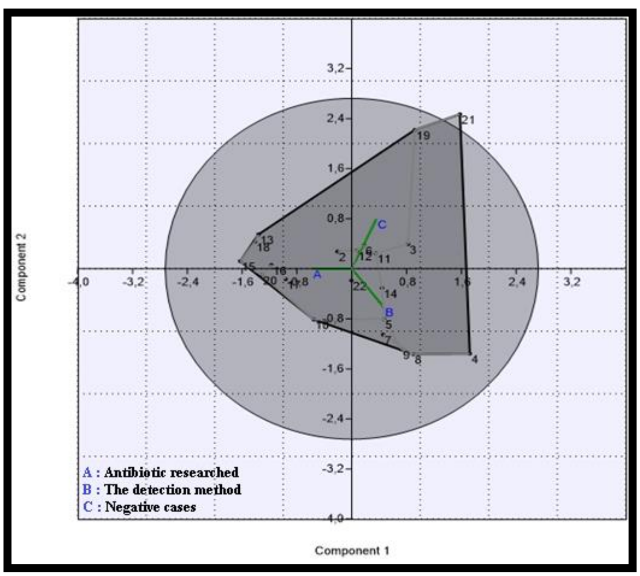

Figure 5. Factors affecting the precision of antibiotic residue detection used in poultry farming in different studies in developing countries (Analysis of the main compounds)

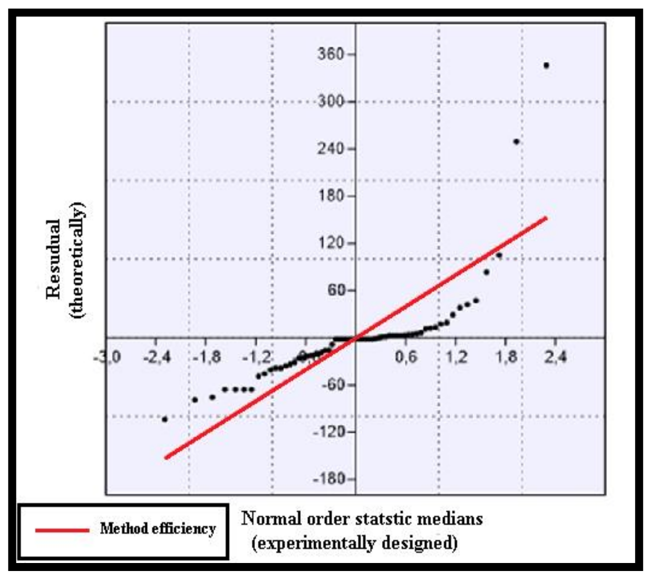

Figure 6. Effect of the Efficiency of the method in the detection of antibiotics used in poultry farming and the accuracy rate (Analysis of residual and normal order)

To properly compare the results for the two classes (positive and negative), The first step consists in developing a regression model for only 2 classes on the associated variable (Figure 3). In the second step, regression by Correlated Components is used to predict values based on detection methods to determine which ones are most reliable in the studies performed (Figure 4). Separate models were developed and then the models for the two classes were compared for the ideal explanation.

The use of this two-step method over two classes improves prediction compared to the traditional correlation coefficient method. The comparison between positive correlation coefficient (R^2 = 43%) and negative correlation coefficient (R^2 = 84%) since the variability of actual dependent detection (poultry samples) is explained by the variable 'detection methods' although both cases are statistically significant (R^2 = 0.84 and p=0.05**)**, for the confirmation of this hypothesis it is necessary to analyze the main compounds statistically (sample size, type of antibiotic sought and method used for detection).

The interpretation of the statistical matrices (Figures 5 and 6) shows that the detection method is the impact factor on the results, for this purpose it can be said that the methods used by the authors are not generally reliable and that the sensitivity of the techniques influenced. Through the results of these previous studies, it must be taken into consideration that the correct determination of residues in white meat involves high-precision techniques.

DISCUSSION

Among African countries, the highest percentage of residues was observed in Algeria "Souk Ahras" (100%), while the lowest in Congo (10.7%). (Okombe et al., 2016; Berghiche et al., 2018). In Asian countries, a high residue rate was detected in Gaza (88%), while the lowest in Iraq (42.5%), while in Europe, the presence of residues was reported lowest level in Bulgaria (15.8%). (Al Pavlov et al., 2008; Shamsa, 2013; Elmanama and Albayoumi, 2016).

The high percentage of positive cases is due to non-compliance with waiting times, as well as self-medication of animals by farmers, who do not know the conditions and doses administered. This may be due to the misuse of antimicrobials probably related to animal treatment followed by insufficient withdrawal time (Corpet and Brugere, 1995; Bonfoh, 2003). Contamination of food of animal origin has been reported by many authors. Indeed, in Algeria, numerous studies have reported the presence of residues, in the Tizi Ouzou region according to Hakem et al. in 2015, sample analyses revealed the presence of 124 positive samples out of 145 collected, representing a percentage of 86.2%. In the same region, Ramdane (2015) reports a 62.5% positive sample rate. In the El Taref region, Mansouri (2007) found that 65.7% of his samples containing antibiotic residues. In the Algiers region, according to Ben Mohand (2007), 3.33% of the samples analysed contained residues of white meat.

A study conducted in northwest Algeria (Benghalem et al. (2016) found that 32 samples of chicken meat from three different regions (Tlemcen, Ain Témouchent and Sidi Bel Abbes) were positive, representing 64% of the total. At the international level, the problem of residues of veterinary drugs in foodstuffs of animal origin, particularly chicken meat, is a real problem. In Senegal, Bada-Alambedji et al. (2004) found 4 positive samples out of a total of 41 samples surveyed, representing a percentage of 9.8%. Two other studies conducted in Senegal (Dakar) by Abiola (2005) and N'kaya (2013) revealed positivity rates of 50.5% and 20% respectively. In Ankara, according to Er B1et al. (2013) 45 ,7% of the samples analysed were contaminated by residues. In Egypt Karmi (2014) revealed 42 positive samples out of a total of 50 samples analysed, i.e. a percentage of 83.33%. In Tunisia Rezguie (2009) found 2 positive samples, i.e. a rate of 16.66%. Two studies conducted in Nigeria by Omotoso (2015) and Ekene (2014) found the presence of antibiotic residues with positivity percentages of 55% and 60% respectively. In Khartoum State (Sudan) (Hind et al., 2014) revealed that 27% of samples were positive for the presence of antibiotic residues. A study conducted in Morocco (Chaiba et al., 2017) on a total of 50 poultry meat samples revealed that 18 samples were positive for the presence of antibiotic residues, representing a rate of 36.15%. Another study conducted in Palestine by Elmanama (2016) revealed 88 positive samples out of a total of 365 samples analyzed, a percentage of (24.1%).

In Bulgaria (Al Pavlov et al., 2008) found that 15.8% of samples are contaminated with antibiotic residues. Two other studies conducted in Iraq by Shareef et al. (2009) and Shamsa (2013) reported positive rates of 52% and 42.5% respectively. In Congo (Okombe, 2016) revealed that 7 positive samples out of a total of 67 samples of chicken meat tested, i.e. a rate of 10.44%. Depending on the method used, antibiotic residues were detected in 944 (37.38%) of the cases by a microbiological inhibition method, including 660 (69.91%) cases using the standard four-boxes method as the experimental protocol.

Four-boxes method has the advantage of being sensitive, with inexpensive reagents, and easy to carry out, because it allows the detection of the following four families of antibiotics, betalactamines and macrolides, betalactamines and tetracyclines; sulfamides and aminosides.

The other advantage of four boxes method is that it is less restrictive and less expensive than other microbiological methods. From our results the most important points that we have noticed, the detection of antibiotic residues in chicken meat requires firstly a high precision technique, a good understanding of the antibiotics marketed in the region that will be studied and a representative sample of white meat for a correct estimation.

CONCLUSION

The application of meta-analysis in biological domains becomes a qualitative leap, where this statistical technique can give reliability and meaning in life science. Despite the fact that the meta-analysis requires a lot of time and that this technique is very complicated compared to reviews. The results obtained from our meta-analysis showed that the precision of the technique for detecting antibiotic residues in chicken meat is the key factor in determining the validity of the results and the strength of its authenticity, even though the variations in the data analyzed, the application of meta-analysis allowed to summarize the results and identify defects in the detection techniques, which encourages scientists in this field to apply it to other topics.

DECLARATIONS

Competing interests

The authors have no competing interests to declare.

Consent to publish

All authors gave their informed consent prior to their inclusion in the study.

Author`s contributions

Berghiche wrote the paper, created the idea and the design the study, Labiad and Berghiche collected data, Berghiche achieves all statistical analysis and drafting of the manuscript. Khenenou and Berghiche read and approved the final manuscript.

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