SEEK BY THE MICROBIOLOGICAL METHOD OF THE ANTIBIOTIC RESIDUES IN DRIED MILK IMPORTS INTO ALGERIA

Bachir-Pacha M.[1], R. R. Triki-Yamani[1], O. Malki[2]
[1]*Institute of Sciences Veterinary surgeon-University S. Dahlab-Blida*
[2]*Veterinary laboratory of D.B.K*

Abstract. The presence of ATB residues in milk powder is the cause of the inhibition of bacterial fermentative processes in the dairy industry. Of the 175 samples of milk powder imported by Algeria and subjected to the acidification test and then to the agar diffusion test, 95% of the tested milk showed contamination. Penicillin and/or tetracycline residues were detected in 64.6% of cases and those of macrolides and / or aminoglycosides, sulfonamides and / or chloramphenicol were detected in 44.3 and 38.3%, respectively.

Keywords: Residue dried milk of ATB, Controls, microbiological Method, Algeria.

INTRODUCTION

Milk is a food adapted to the nutritional and physiological needs for all the ages of the life. For the greatest number of the population, milk is a product accessible by its price and, consumed to make up the animal protein deficit and to ensure a more or less balanced food intake.

In Algeria the production of milk does not meet the needs for the consumers, which implies the importation of significant amount of dried milk and dairy products (1.8 billion $ in 2014). These problems locate the limits of the development of the die which is dependant by its raw materials and its technologies on outside. It is thus regulated by the State. In addition to its nutritional and organoleptic qualities, to be specific to human consumption, milk must be before a whole product healthy, i.e. of pathogenic germs, but also free from residues of drugs. The antibiotic residues in dried milk imported must be a source of concern of the public authorities, especially when one knows their harmful effects on the human health (antibiorésistance, allergic problems), on dairy technology (transformation of the dairy products), on the nation's economy and during bacteriological controls (wrongfully negative results).

The analysis of this foodstuff is necessary in order to guarantee the control of the quality (conformity) from a medical or purely commercial point of view. The importance of this problem of a medical and economic nature and the scientific lack of documentation on the question of the residues in our country guided us in the choice of this topic.

MATERIAL AND METHODS

Our study was carried out on 04 types of dried milk been essential (whole, skimmed, half-skimmed and instantaneous) to detect the antibiotic residues by the official microbiological method of the French agency of public health of the food **[1]**. The analyses related to 175 samples made up of:

Type of dried milk	Many samples	Rate M.G
Entirety	75	26%
Skimmed	50	0,26%
Instantaneous	40	26%
Skimmed half	10	14,5%

These dried milk samples imported of France, Zealand News, Northern Ireland and Argentina, come from the veterinary inspection of the stations frontier of Bejaia and analyzed at the regional veterinary laboratory of Draa Ben Khedda (Tizi Ouzou). Two techniques are successively implemented:

Test of acidification: it rests on the description at the time of presence of residues of ATB, an inhibition of *Bacillus stearothermophillus* variety *calidolactis* ATCC10149, indicated by the turn of an indicator.

Diffusion on gélose (Test of confirmation) with 03 stocks of *Bacillus*

(B. stearothermophillus ATCC 10149, *B. subtilis* ATCC 6633 and *B. megaterium* ATCC 6618.

This test follows limits of detection (Table 2). After 24 hours of incubation the diameters of the zones of inhibition are measured using a metal slide caliper.

RESULTS

1 Test of acidification. From the total of the tested samples, the turn with the color crimson of the blue of bromocrésol was noted on 160 samples (92%), translating the presence of residues of ATB. However, 07 samples (4%) are doubtful (turn with the gray) (Table 1).

Table 1

Results of the Test of acidification

Types of dried milk	Total analyzed Samples	Test of acidification in %		
		Negative	Doubtful	Positive
Entirety	50	0	0	50 (100%)
Skimmed half	10	0	0	10 (100%)
Skimmed	75	0	7 (10%)	68 (91%)
Instantaneous	40	8 (20%)	0	32 (80%)
Total	175	8 (5%)	7 (4%)	160 (91%)

2 Test of confirmation. The diameter (Ø) of the zones of inhibition formed around the discs of impregnated sterile filter paper of milk to test and the ATB pilot was measured: Positive = Ø ≥ 8mm & Negative = Ø < 8 Misters Out of the 175 dried milk samples tested with *B. subtilis, B. stearothermophilus,* and *B megaterium,* 167 samples (95%) are positive, and the 08 remainders are negative (5%).Among the positive samples we revealed the presence of residues of ATB: 18 with Penicillin-Tétracycline (65%), 74 in Macrolides-Aminosides (45%), 64 with the Chloramphenicol-Sulphamides (38%), 16 with Penicillin-Tétracycline and Macrolides - of Aminosides (10%), 09 with Penicillin-Tétracycline and Sulphamide-Chloramphenicol (5%), 16 in Macrolides-Aminosides and Sulphamide-Chloramphenicol (10%), 29 in Macrolides-Aminosides, Sulphamide-Chloramphenicol and/or Penicillin-Tétracycline (17%).

Table 2

Total results of the tests of confirmation: percentage of the positive cases

TPE	Positive samples (%)	Residues found according to the families of antibiotiques%						
		Pn/Tt	Mc/Am	Sd/Chp	Pn/Tt/Mc/Am	Pn/Tt/Sd/Chp	Sd/Chp /Mc/Am	Mc/Am /Sd/Chp / Pn/Tt
	100	60	42	46	6	10	6	18
LDEC	100	20	20	40	0	10	0	0
LÉC	100	68	48	48	13,3	5,3	16	14,7
LI	80	78	47	34,4	9,4	0	0	28,1
Total	95	64,6	44,3	38,3	9,6	5,4	9,6	17,3

TPE: Type of dried milk; **:** Full-cream milk; **LDEC:** Skimmed half milk; **LEC:** Skimmed milk; **LI:** Instantaneous milk.

Pn/Tt: Penicillins and/or Tétracyclines; Mc/Am: Macrolides and/or Aminosides; Sd/Chp: Sulphamides and/chloramphenicol; Pn/Tt /Mc/Am: Penicillins and/or Tétracyclines, Macrolides and/or Aminosides; Pn/Tt/Sd/Chp: Penicillins and/or Tétracyclines, Sulphamides and/chloramphenicol; Sd/Chp/Mc/Am: Sulphamides and/chloramphenicol, Macrolides and/or Aminosides; Mc/Am /Sd/Chp/ Pn/Tt: Macrolides and/or Aminosides, Sulphamides and/chloramphenicol, Penicillins and/or Tétracyclines.

DISCUSSION

The test of acidification initially made it possible to carry out a screening of dried milk samples tested (175) and to obtain 160 positive (91,4%) and 8 negative (4%). This test did not make it possible on the other hand to conclude on the seven doubtful samples (4,6%). The rate of positivity of 100% was reached by skimmed dried milk whole and half, of 91% for skimmed dried milk, of 80% for instantaneous dried milk.

The test of acidification makes it possible to detect the maximum of substances to a close threshold, even lower, with their LMR. It makes it possible to analyze a great number of samples in a more or less fast time [7]. As for the test of confirmation, it makes it possible to determine, according to the medium and of the micro-organism, the presence of residues of ATB of the various families.

The use of the discs of pilot ATB for each medium enabled us to check the reliability of the technique i.e. the good concentration in micro-organisms test and the sensitivity of the latter with respect to the ATB, which makes it possible to deduce that the operating conditions are credible. The presence of zones of inhibition is due to the presence of residues of ATB in the analyzed sample, which migrated in the medium, thus inhibiting the growth of the microphone-organization-test around the discs impregnated of milk samples to be tested.

The technique of diffusion in gélose (test of confirmation) made it possible to confirm the positivity of the 160 samples and raising any ambiguity on the positivity of the 07 doubtful samples, which brought back the rate of positivity to 95%. The revealed negative samples and confirm by the test of confirmation express the reliability of the test of acidification. The positive doubtful samples revealed by the test of confirmation, can be explained by the presence of a concentration of ATB higher than the threshold of detection of the germs test. One then obtains a zone of inhibition higher or equal to 8 Misters.

The test of confirmation with *B. stearothermophilus,* revealed that 108 samples (64,6%) of a total of 167 presented zones of inhibition annular higher than 8 mm, which have the highest rate. These samples were regarded as positive for the residues of penicillin and/or tétracyclines.

The test of confirmation with *B. subtilis* revealed that 74 samples (44,3%) of a total of 167 also introduced to zones of annular inhibition higher than 8 mm ± 6,34 Misters These samples are positive for the residues of macrolides and/or aminosides.

The test of confirmation with *B. megaterium* revealed that 64 samples (38,3%) of a total of 167 introduced to zones of annular inhibition higher than 8 mm ± 6,34 Misters These samples are positive for the residues of sulphamides and/or chloramphenicols.

The test of confirmation with the various stocks test revealed us that certain samples are positive for two or several families of ATB: Penicillin and/or Tétracycline and Macrolides and/or of Aminosides (9,6%): Penicillin and/or Tétracycline and Sulfamides and/or Chloramphenicol (5,4%): Macrolides and/or Aminosides and Sulfamides and/or Chloramphenicol (9,6%) and, Macrolides and/or Aminosides, Sulphamides and/or Chloramphenicol and/or Penicillin and/or Tétracycline (17,36%). These results confirmed the dominating place occupied by penicillins and the tétracyclines in the therapeutic one in dairy breeding in the world. Moreover, these results confirm the effect of the policies followed by certain countries (such as France) with respect to chloramphenicols and of the sulphamides (strict prohibition of use in dairy breedings).

The positive samples simultaneously for several families of ATB, indicate that the animals received several families of ATB (association), whereas the association of more than two ATB is strongly disadvised. It nevertheless is used either on a purely curative basis, or preventive (promoter of growth in supplementation in food). The highest rate of positivity of the dried milk samples whole, skimmed and instantaneous, was for the residues of penicillins and/or tétracyclines (respectively: 60%,68% and 78%). These results reiterate that these families of ATB (penicillins and tétracyclines) are used in breeding dairy bovines in the world.

According to Sanders (2005), the milk containing of the residues of ATB is a bad culture medium for the lactic bacteria used in industry; the starting of their growth is often delicate. The micro-organisms used in the manufacture of the dairy by-products are sensitive to the inhibiters and this, according to the nature of the ATB and the leaven species. They are for the majority sensitive in the majority of the ATB used in veterinary medicine, as private individuals with penicillins. This explains why residues ATB, even with weak concentration, can involve their inhibition (for example if milk contains penicillin residues with 0,50 UI/ml, it involves a total inhibition of the lactic leavens). It was shown that 0,1 and 0,05 unités/ml of penicillin residues slowed down the production of lactic acid inducing the deterioration of the quality of produced cheese.

It is thus plausible to affirm that the main cause of certain incidents in cheese dairy is due to the inhibition of the lactic leavens because of presence of penicillin residues in dried milk imported and used like raw material. Milk coming from treated animals is not that seldom isolated collection because of nonthe respect of the withdrawal periods necessary after use of the ATB [8]. The heating of milk makes it possible to eliminate part of the residues; but all the ATN do not have the same thermolability [7]. That explains the found results. Thus, the heat treatments and the drying of the milk of origin made it possible to eliminate only one small fraction from the residues of ATB of origin. The consumption of the dairy products resulting from these powders can then involve harmful effects for the

public health, and that even if the raw material undergoes heat treatments (pasteurization, sterilization) to be transformed into one or more dairy by-products.

It would also seem that the presence of ATB in dried milk is not due solely to residues of treatments managed with the animals and nonthe respect of the withdrawal period. That can be also with fraudulent treatments by voluntary addition of ATB in milks for a better conservation [5].

The microbiological method of analysis implemented and using *B. stearothermophilus* like micro-organism test, is characterized by a threshold of detection of the ATB to nearest to the maximum limits of residues (LMR) of the ATB most frequently used in the treatment of the dairy bovines while preserving a broad spectrum of activity. Indeed, the sensitivity of the novel method of acidification is much better for the two families of ATB most largely found in the whole of the samples tested (beta-lactam antibiotics and tétracyclines). *B. stearothermophilus* is characterized indeed by a notable sensitivity to the β-lactamines, its growth is inhibited by a concentration of 5 ampicilline Pb [3, 9].

Only the results of this test of confirmation are officially taken into account. It makes it possible to officially sanction a stockbreeder for a "inhibiting accident", with repercussions during the payment of milk to quality [7].

Few studies concerning the presence of antibiotic residues in dehydrated milks were carried out in Algeria. The only point of comparison is compared to a study undertaken in 1989**,** by Lebres E. and Moufouk F. on the search of ATB and residues of ATB in imported dehydrated milks. The results show that on a total of 136 treated milk samples, 33 (25%) present residues of ATB and 103 (75%) are negative, definitely lower than our results (95%). These differences are primarily explained by the place increasingly important catch by the ATB in the breedings on a purely curative and preventive basis and as a food additive in the drieds milk (end product) to increase their shelf life.

The presence of residues of ATB in addition to the direct or indirect risks relatively moderate that they present for the consumer [2, 10,11] can appear a true plague for the processing industry of milk, in particular for the manufacture of cheeses and yoghourts. They are at the origin of inhibition partial or total of the phenomena fermentaires of bacterial origin. These technological consequences depend primarily on the residual amount of inhibiters in milk and on the sensitivity of the lactic germs used to the ATB [4, 6]. It consequently becomes obvious that the inhibiting residues should constitute a constant concern of the industrialists of the die milk in Algeria, from the qualitative and quantitative depreciation of the dried milk which they are essential and even dairy production that they induce. Consequently, the installation of a regular control system not to say systematic milk intended for the transformation consequently becomes pressing.

In spite of its qualitative aspect, the search for residues of ATB by the microbiological technique remains a technique simple to implement and inexpensive compared with the immunological and chromatographic techniques whose cost considerably higher would certainly not allow their generalization within the framework of dairy control in Algeria.

CONCLUSION

All the families of ATB tested in the various types of dried milk imported are present and, atdefinitely high rates. The presence of residues of ATB in milk, expresses the width of unreasoned use of the ATB in the treatment of the dairy animals and nonthe respect of the withdrawal period even, the voluntary addition of the ATB in dried milk to prolong its shelf

life. Taking into account the results obtained and risks on the health of the consumers and the milk industries that the presence of residues in dried milks confers imported, one could recommend:

To set up a plan of monitoring to in general control the presence of residues of ATB in milk and it dried milk imported in particular.

To sensitive, the various actors of the die (stockbreeders, importers and persons in charge) of the control of the foodstuffs of animal origin in particular milk, and to incite them to work of coordination for better protecting health from the consumers.

To improve and increase the local production of milk, and to better manage its quality by the analysis of the possible hazards related to a product or a process, which must then be applied to the whole of the die of "the *cattle shed with the table*".

Lastly, it would have been desirable to supplement our work by a quantitative study (high performance liquid chromatography = HPLC), more significant to refine the results of the concentrations of residues of ATB present in the analysed dried milk samples, because the gravity of the risks is dependent on these contents.

REFERENCES

1. AFSSA, 2006. Veterinary use of antibiotics, bacterial resistance and consequences for the human health. Arranges French Public health of Food, p. 214.
2. Dewdney J.M., L. Maes J.P. Raynaud, F. White J.P., Scheid T. Jackson S., Lens and C.Verschueren, (1991). "Risk assessment off antibiotic residues off β-lactams and macrolides in food products with glance to their immuno-allergic potential". Review: Food and Chemical Toxicology*",* n°29, p477-483.
3. Heeschen W.H., A. Bluthgen G., Johnson H., Charm and Zomer E., (1991). "Detection and confirmation off inhibiters in milk and milk products". Bulletin off the International Dairy Federation. p. 258.
4. Labie CH., (1981). Legislative measures intended to avoid the presence of antibiotic residues in milk. Review: collection of veterinary medicine, n°157, 161-167.
5. Lebres E. and Moufouk F., (1989). Research of antibiotic and the antibiotic residues in milks. Review: The VETERINARY MAGHREB, vol. 4, n°17, 5-7.
6. Mitchell J. Mr., Griffiths Mr. W., McEwen S.A., McNab W.B. and Yee A.J., (1998). "Antimicrobial drug residues in milk and meatus: causes, concerns, prevalence, regulations, tests, and test performance". Review: J. off Food Protect, n°61, 742-756.
7. Sanders P., (2005). The antibiorésistance in veterinary medicine: public health issues and of animal health. Review: Veterinary bulletin of the academy of France, volume 158, n°2, p. 139.140.
8. Sraïri m.t., Hasni I., alaoui A., Hamama, Faye B., (2004). Physicochemical quality and contamination by antibiotics of the milk of mixture out of intensive cattle sheds in Morocco. Review: Renc. Rech. Ruminants, n°11, p. 116-117.
9. Suhren G., Heeschen W., (1996). "Detection off inhibiters in milk by microbial tests. Review: Nahrung, n°40, p. 1-7.
10. Sullivan A., Edlund C. and Northern E.C., (2001). "Effect off antimicrobial agents one the ecological balances human off will microflora". Lancet Infectious Diseases, n°1,101-114.
11. Wal J. Mr., (1979). "Evolution off the concept off residues in the products off animals raised with the uses antibiotics off". Review: Annals of the Nutrition and the Food, n° 33, p. 325-341.