

Evolution of Food safety and microbiological methods: a few considerations and comments

Cécile LAHELLEC , Honorary research director, French Food Safety Agency

(cecile.lahellec@sfr.fr)

X workshop on Rapid Methods and Automation in Microbiology

Barcelona. November 22/10/2011

It is my very great pleasure to be here today and I would like to thank very warmly Marta and Josep for inviting me to present the introductory paper for this Xth anniversary of the Spanish workshop on Rapid methods and Automation in Food microbiology .

The title of my presentation is " Evolution of Food safety and microbiological methods : a few considerations and comments " .

The reason of that choice is as follows : I think that we are always at one period of history , whichever the field we consider and it is always uneasy to be conscious of the fact that the period we live is the result of many discoveries, many efforts and events which occurred previously . That is particularly true in the field of Food safety in which we are interested today .

The problems of Food safety are not new ; they have occurred from a very long time and , at each period of our history , men tried to face problems ; but, of course, the way of thinking has been submitted to a lot of changes and evolutions . That is the reason why, in the first part of my presentation, I shall comment some crisis described in the early Food safety history before the time when the role of some particular agents in Food safety problems was pointed out .

The discovery of microorganisms and of their incidence in food has been an important step in the origin of different diseases obviously related with food ; however, Food microbiology found its own identity after the 2nd world's war only . That will be the second part .

The microbiological methods said " pastorian" were used all around the world and are still used as " reference" methods ; but the face of Food microbiology as completely changed with the development of rapid methods and automation in microbiology and, especially, with molecular biology approaches ..

Due to the necessity to be accepted officially in international trade , those methods had, of course, to face different difficulties ; I shall mention those related to their acceptance by standardization bodies .

Then, after a few considerations and comments on changes observed within the types of foods and ways of eating during the past decades I'll describe shortly , in the last part, the new scientific context of Food safety and the role of Food safety agencies ; we shall examine till which extent the improvement of microbiological methods may help our understanding of some Health problems related with Food .

Finally , I'll draw a short conclusion .

I- FOOD SAFETY : what was it a few centuries ago ?

The concept of Food safety is not a new one .

From the beginning of humanity, food had some sacred meaning and, for that reason, it has been considered that eating must not lead to any health problem .

We all know that, even nowadays , some types of foods, like pork, are still submitted to religious prohibitions (for Jews and Muslims especially) even if they are no more in relation with any concrete health problem .. they have anyway to be considered as prescriptions written in the Bible or the Coran .

In fact, from a long time , food has been incriminated in problems of illness but the origin of contaminations was not known . One of those crisis , related to bread was described firstly by Ademar de Chabannes in 997 . The illness was very severe and a lot of people died from in the center of France ; however , people were very poor and they had to make a cruel choice : eat the contaminated bread or not to eat at all and die ... Most often , Food security problems had to be taken under consideration before those of Food safety .

The agent of the disease, *Claviceps purpurea* , was identified , at the beginning of the 19e century only , by a botanist called Candolle .

When we have the opportunity to learn a little about the history of fears resulting from the ingestion of food (a very interesting book is that of Madeleine Ferrières published in 2002), we are conscious that, during

many centuries, even if the situation was very different from now as the existence of microbes was not known, different approaches concerning the risks related to food were undertaken. From different examples, we can foresee what is called now "Risk Evaluation"; different groups of people: scientists, experts, profane, may be consulted to give their advice and try to find a consensus; then, the decision may be taken by a magistrate and, sometimes, somewhat different from that of the experts ...

II- THE DISCOVERY OF THE EXISTENCE OF MICROORGANISMS :

has opened a decisive step in the comprehension of the origin of food diseases. However, we have to keep in mind that, by intuition, empirically, men were conscious of the presence of microorganisms in food. As an example, we know that there was a brewery in Babylona, most probably 5 to 7 thousand years Before Christ. We know also that salt from the Dead Sea was used to preserve Food, that Babylonians and Chinese even prepared fermented sausages 1500 years BC .. They knew what they could do but they did not know why they were so successful.

Many centuries passed along: according to James M. Jay, 1978, in "Modern Food Microbiology" "the first scientist who suggested the role of microorganisms in food was probably A. Kircher who, as early as 1658, observed decaying bodies, meat, milk and other substances and saw what he referred to as "worms" invisible for the naked eye. It is then, possible to take under consideration the works of L. Spallanzani (1765), who observed that beef broth submitted to boiling for one hour and sealed remained sterile but he did not succeed in convincing his colleagues because the treatment to which the broth had been submitted excluded oxygen. A lot of researches were carried out consequently. We can remember here especially, the beginnings of canning; in 1795, the French government offered a prize of 12000 fr for the discovery of a practical method of food preservation. In 1809, a Parisian confectioner, François (Nicolas) Appert could preserve meats in glass bottles that had been kept in boiling water for varying periods of time. This discovery was made public in 1810 when Appert was issued a pattern for his process. Not being a scientist, Appert was probably unaware of the long-range significance of his discovery or why it worked. This is, of course, the beginning of canning as it is known and used today. This event occurred some 50 years before Louis Pasteur demonstrated the role of microorganisms in French wines, a development which gave rise to the rediscovery of bacteria (they were seen under a microscope and described by A. Leeuwenhoek in 1883 but it is unlikely that Appert was aware of this development since he was not a scientist and Leeuwenhoek's report was not available in French. The first man to appreciate and understand the presence and role of microorganisms in food was Louis Pasteur who, in 1837, showed that the souring of milk was caused by microorganisms; he employed for the first time the use of heat to destroy undesirable microorganisms in wine and beer (pasteurization)".

III- THE EVOLUTION OF MICROBIOLOGICAL METHODS

The first real developments in Food microbiology can be observed after the second World war only: as nowadays, the evaluation of Food safety always began by a visual inspection and microbiology was used as a help to the decision

The microbiological techniques used, which, in France, were taught from the early fifties by R. Buttiaux (who was a physician), in the Pasteur Institute in Lille (F) derived from the discoveries of Louis Pasteur. I was lucky to follow the courses in 1967 and 1968. If I remember well, in May, each year, it was possible to learn through theoretical and practical work all bases of Food microbiology; four courses were organized: one on water, one on meat and derived products, one on milk and dairy products, the last one concerned all other types of food products (fish or poultry, for example). I still have those courses in mind as Pr Buttiaux had never any paper to help him during his presentations. From that time, he taught us about the ecological development of microorganisms; concerning the practical work, he was very keen of the way we were conducting the manipulations and, to help us, we could follow on a screen all developments; we had also to know by heart the characteristics of microorganisms; we were conscious to learn the real basis of Food microbiology ... Those techniques are the conventional ones used at reference methods even if, of course, a lot of improvements have been afforded, new media have appeared on the market, a lot of specifications have been given in the purpose of accreditation, as we'll see later, new technologies may be introduced in some cases (It has to be

mentioned that, In France , the first regulation giving microbiological criteria , published 21st December 1979 , gave criteria and the related microbiological conventional methods). Quite at the same period , due to the necessity of examining a sufficient number of samples , representing as closer as possible the population , many scientists began to think to alternative methods which may be used in laboratories in order to control and maintain Food safety in most countries . The first scientist to propose practical solutions is somebody who, providentially, is among us today . and I like to tell you a story I repeat each year :I was participating in a symposium in Kiel(D) in 1974 . I can't forget it.. I did not present anything on that day ; I was just learning and listening at the different papers when , suddenly , we had a very interesting and unusual presentation : the speaker , a young American Chinese was quite "dancing" , showing beautiful slides : the miniaturized methods used to detect or enumerate microorganisms from poultry was the subject of his presentation : he was using microplates instead of tubes and a micro-inoculator instead of platina loops ...He had a very simple principle" THINK SMALL" . As I was working on poultry meat microbiology and was very much interested by rapid and economic methods in order to study a large number of strains simultaneously , I met Dr Fung at the end of the session , asking for informations and reprints ; I must say it was the beginning of a long story .. first because he sent me reprints and even his thesis and, from that time we became very good friends but I must say, too , that it opened me new horizons , new contacts and a willingness to communicate about the possibilities which were offered to food microbiologists: they could use rapid method which were , till that period, used in the medical field only ... for me , it was the beginning of a long story in science and friendshipDr Fung visited our Institute in 1976 and , from that time , miniaturized methods were introduced in our laboratory and used to identify a lot of strains to trace *Salmonella* , then different types of pathogenic microorganisms " from farm to fork"; we were so enthusiastic with those methods that we presented the technique as well as the results during different meetings in different laboratories : we wanted those methods be adopted by the scientific community ... and were somewhat successful .In fact , Dr Fung is really the "father" of all the commercial methods of identification which appeared on the market from the seventies and I must say I have been very lucky to stay in his laboratory during 2 months in 1980 . Simultaneously , different scientists around the world began to think to new techniques derived from different branches of Science ; in fact , the microbiological controls taking place in industrial laboratories,they had to find a way in order to :

- Get a rapid answer in order to cancel any abnormality in processing food and, by that way, avoid to put on the market some products which would not fill regulatory standards or standards proper to the concerned industry
- Low cost in order to allow a high number of samples to be examined ,

All that being realized , keeping in mind the results have to be accepted by the scientific community .

In that context , many efforts have been proposed quite early , in order to set up methods based on the following principle : the microbial population is evaluated by detecting a signal related with the activity of microorganisms , most often an enzymatic or the concentration of a molecule (coenzyme, metabolite) or a change appearing in the medium (pH or Redox potential impedance, heat production variation) in connection with that activity . Radiometric methods were proposed and studied by P.Mafart in Quimper .

A lot of methods based on immunology have been developed but it seems that, during the last decade , the most spectacular developments have concerned the introduction an use of methods based on molecular biology , including now DNA chips , which means a fantastic technological evolution, for the characterization of strains ... During the week , you will be lucky to learn a lot about the evolutions but one of the difficulties, of course, has been to make the commercial techniques accepted for official controls . That is the reason why I would like to share with you an example of the difficulties encountered to introduce new technologies in regulations ...

IV- THE LONG WAY FOR THE OFFICIAL ACCEPTANCE OF ALTERNATIVE MICROBIOLOGICAL METHODS

The problem of acceptance of alternative microbiological methods has been thoroughly studied during the past years . and somewhat solved , due especially to the actions undertaken in ISO and CEN meetings . Those two organizations will be described shortly in order to better understand the context.

ISO (International Standards Organization) is the International Standards Organisation ; this organization was created in October 1946; its seat is located in Geneva (CH) ; the creation results from the fusion of two organizations :

ISA which was the International Federation of National Associations of Standardization, founded in New-York in 1926 and

UNSC, i.e Committee for coordination of standardization of United Nations, created in 1944 .

The first national Assembly was held in 1949 in the great amphitheater in Sorbonne (Paris). ISO is composed of 247 committees . All standards are obtained by consensus ; however , they are not mandatory ..The technical committee in charge of Food microbiology is TC34 / SC9 ; the actual president is Bertrand Lombard (F); the meetings are held in a different country each year ; for example , in 2009, the meeting was held in Valencia (Spain) ; In June 2011 , the meeting was held in Bournemouth (UK) ; as usual, it was a joined ISO/CEN meeting

CEN, Comité Européen de Normalisation ("European Committee for Standardization ") has been created in 1961 in order to harmonize the standards elaborated in Europe ; that means that the standards are mandatory in all countries of EC (at the contrary, the standards which are elaborated by ISO are facultative , which means a great difference ... All members are members of ISO as well .

The seat of CEN is located in Brussels (B).In the beginnings , it was created by the national organisms for standardization from France, Germany and Benelux countries .Nowadays, the full members are the 27 countries of EU and the three contries of AELE (Association Européenne de Libre Echange) which own such an organism (Switzerland, Norway and Iceland). CEN elaborates technical standards in favor of international trade .

CEN/TC275/WG6 was created in 1993 and I was nominated as the convenor ; nowadays , from July 2005, Alexandre Leclercq from Institut Pasteur in Paris , is in charge of the group .

From the beginnings, one main principle has been followed during the work of this group , i.e the Vienna Agreement ; this agreement requires that, as often as possible, ISO methods are taken In order to avoid any overlap, there is also an agreement between different groups of CEN that only one group is in charge of a particular method (the " Vienna agreement") . For example, TC302 in charge of milk and dairy products analysis may choose one specific technique . In this case, it requests TC 275/WG6 to refer to this specific technique in the standard method . The necessity of taking into account the experience of other groups around the world, for example AOAC and IDF (International Dairy Federation) , has also been emphasized from the beginning and , presently, the basis for a good cooperation has been set up .

In order to maintain a good international cooperation, one part of the meeting concerns the liaison with other organizations , ie : International Dairy Federation (IDF) Codex Committee on Food Hygiene , AOAC (Association of Official Analytical Chemists), WHO (World'Health Organisation) , IUMS (International Union of Microbiological Societies) . The necessity of getting an international consensus , especially with Codex alimentarius , is always kept in mind.

Due to the evolution of techniques proposed, one important problem discussed during many ISO /CEN meetings has been the introduction of new technologies in Food microbiological methods and , to finalize the project, it took a very long time ...

Finally, an important resolution was taken during the joined meeting held in Parma (It) in April 2004.

. " *Each time a standard method is being revised , the possibility of using new technologies , including PCR, must be examined by comparing results with those obtained when using the official conventional method .*

- *For a given microorganism, in order to complete the existing method, the development of standardised methods based on new technologies can be proposed when the purpose to be obtained (for example pathogenicity level) makes it necessary.*
- *When new technologies, including PCR, are used as alternative methods, they must be validated against the reference method.*

Those sentences look probably as quite simple, but I am sure you cannot imagine the number of hours of discussions which were necessary to obtain a consensus: that is "international cooperation"

But the hours of discussions were very fruitful..as, finally, rapid methods were accepted according to the EC regulation 2073 15 December 2005 concerning microbiological criteria: ***"Test results are dependent on the analytical method used and, therefore, a given reference method should be associated with each microbiological criterion. However, Food business operators have the possibility to use analytical methods other than the reference method, in particular more rapid methods, as long as the use of these alternative methods provide equivalent results"***

So, alternative methods were finally, officially accepted in the EC, which is surely an important step in the new context of Food safety as a lot of controls and investigations have to be realized.

V- THE NEW CONTEXT OF FOOD SAFETY

The new context which will be described shortly thereafter has taken place in the early 1990's after different Food crisis which may be explained through different reasons:

The types of foods we eat is very different of that we ate 50 years ago; and a lot of microbiological changes may be observed according to the food, the conditions of preparation, of storage... As an example, I still remember the time when crude vegetables, as minced carrots, appeared on the market; a lot of controls began which showed the presence of *Yersinia* (including some *Yersinia enterocolitica*), and some types of fears suddenly appeared; at that time, they concerned more the scientists than the consumers who were not really informed of any eventual, potential danger. At that time, even if some familial outbreaks were published, the situation was really very different from now...

There are also large changes concerning the origin (different types of meats or vegetables...etc, come from different parts of the world); moreover, different meals are prepared in a central place, then distributed around... That explains the contamination of a lot of people from a unique preparation.

Those changes have led progressively to large outbreaks which have been known from the consumers. Nowadays, each incident seems to be published immediately in the newspapers, and that is very different from what we could observe, even 20 years ago. I always remember what I heard in 1992: I was in Washington DC for a Codex alimentarius meeting and I went to a travel agency to change my plane ticket. At the desk, a nice lady asked me: which is the reason of your stay in US? I answered I was participating in the Codex Hygiene and she asked me: are you aware of the problems of *E.coli*. I was really surprised by that question... and I remembered that this year...

In fact, from 1992, we have had to face different problems of Food safety. As an example, we can remember the problems of *Listeria monocytogenes* when the responsibility in different outbreaks have been discovered; then, other foodborne diseases have taken place in the public debate.

The result of those food crisis, which were better and better investigated due to new techniques and new technologies have led, in Europe, to large changes in the apprehension of Food Safety: I want to speak of the creation of Food Safety agencies in quite all European countries and, in 2002, the creation of the European Food Safety Agency. All problems are now investigated according to a protocol of Risk analysis. In order to set up a good Risk assessment necessitates to have a lot of good and representative data – and that is very difficult in microbiology; in that context, the improvement of methods as well as the introduction of predictive microbiology can help but realistic quantitative risk analysis are only a few at the moment... as microbes are alive and can grow and multiply in very different conditions; a lot of analytical problems are not solved, in spite of many improvements; virulence characterization is always very difficult to appreciate and may vary even for strains belonging to a same species; we have also to consider a lot of factors which

may influence the growth or, simply, the survival of microorganisms (pH, temperature, water activity, oxygen, nature of the food and available substrates..); then, we must not forget the individual susceptibility ...A quantitative evaluation of microbial risk will always be very difficult to set up ...
Whichever the context in which we try to put the microbes , they will always make us know their personality!

That seems very important at a period when young microbiologists may forget they are working on microbes and many teachers in universities think that problem has to be seriously taken under consideration . Of course, we have to recognize we are far from the time we were drawing the shape of microbes when looking in a microscope !! Probably, an equilibrium has to be found in order the improvement of techniques does not hidden the real world microbiologists are studying ...

What to say as a CONCLUSION ?

At first , the problems of Food Safety are not new (M.Ferrières asks “ what is new ?“) but, of course, along the centuries , the world has changed (the types of foods we eat are very different from those of the Middle age , or even very different from what we had 50 years ago , the origin of food is worldwide , the way of eating ..) and the way of apprehension of the problems too .

All changes in our environment, our way of living and eating , the type of published informations, have led to radical changes. Different crisis : Listeriosis , BSE have led to a new way of apprehension of the problems and the creation of national Food safety agencies , then of the EFSA (European Food Safety Agency)
And , suddenly , I realize, that , personally , I have been living one important period of the History of Food safety .. and I would like to share with you some comments as a conclusion :

The reason why I have been interested in pathogenic microorganisms as *Salmonella* in the early 70's was the development of further processed poultry products on the market ; at that time , the Ministry of Agriculture was afraid of potential Public health problems due to the contamination of those products ; we were then requested to participate in the elaboration of microbiological criteria ... that was the beginning of the story ; we observed the *Salmonella* contamination of a somewhat high percentage of products and decided to look at the origin . A lot of epidemiological surveys were then realized which made us observe the contamination “ from farm to fork “ ..but the communication was very difficult at that time because there was not any obvious problem of public health .

Later on , the problem of the vertical transmission of *Salmonella* through eggs seemed more important to the public authorities , and especially because it was officially worldwide ..Some physicians were involved in the problem , visiting US as well to try to understand what did happen ; epidemiological surveys were realized and the results were published in professional journals ; more and more people began to be aware of the problems .

Simultaneously, rapid/alternative methods were developed on the market and were more and more often used (the first API galleries were found on the market from 1970) but there was no official acceptance .

Then, from 1992 , the Food safety problems became more and more known from the media when the evidence of foodborne transmission of Listeriosis was shown . At that time , our laboratory was involved in methods of detection ; simultaneously , I participated in meetings organized in Paris by the “ Conseil supérieur d'hygiène publique de France” ; those were the first approaches to the creation a few years later , of the Food Safety Agencies ..

During the same period too, the standardization bodies (AFNOR in France , CEN in Europe, and ISO) were more and more involved in the problems of acceptance of alternative microbiological methods .

I feel lucky to have been involved in all those developments ..

But a long way remains and the improvement of our knowledge in Food safety must not make us forget the topic is a very difficult one . Microbes are alive and will make them know if we are tempted to forget the reality