THE FRENCH NATIONAL NETWORK OF EPIDEMIOSURVEILLANCE OF THE ANTIBIORESISTANCE OF PATHOGENIC BACTERIA IN BOVINES: RESULTS OF A PERMANENT QUALITY CONTROL SURVEY

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The French national network of epidemiosurveillance of the antibioresistance of pathogenic bacteria in bovines was set up in 1982 to control the evolution of the resistance of *enterobacteria* against antibiotics. It was then extented to other bacterial species.

METHOD

The network is based on the principle of the gathering of results of antibiograms established by the Veterinary Laboratories of Analysis; these informations are registered in a computerised database which, for the time being, contains more than 14 000 antibiograms.

In addition to the *enterobacteria*, the germs thus controlled are the Staphylococcus and *Pasteurella*. The antibiotics which are tested are those normally used by veterinary practitioners.

QUALITY CONTROL SURVEY

A permanent quality control survey was set up so as to be able to measure the variability of the method, and to detect any modification within the technics used by the various laboratories involved.

To do so, members of the network use three reference strains which have been selected for their particular stability and representativity: an *Escherichia Coli* strain, isolated from calves feces (diarrhoea); a *Staphylococcus Aureus* strain (bovine mammitis) and the international reference strain of *Staphylococcus Aureus* 7625 from the Institut Pasteur (Paris) collection (Ref. 25923 of the American Type Culture Collection). These strains are kept lyophilized and regularly tested by the laboratories without any imposed timing.

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STATISTICAL ANALYSIS

Only results obtained with the E. Coli 0132 reference strain are considered here.

Mean diameter per laboratory

The results which are summarized in Table I concern 22 antibiotics tested by 22 laboratories. Depending from the laboratories and from the antibiotics used, the number of tests varies from 1 to 100 over a period of time of 7 years.

It is noticed that for some agents of a diameter either very small or very big, results are not very different from one laboratory to the other. On the contrary, data are more spread out when diameters are included between these two extreme values.

Variance Analysis

Results come from 16 laboratories who each made 5 measurements with 5 different antibiotics (Table II). The main effects are studied both according to the laboratory and to the antibiotic tested. The analysis show that the differences owed to the laboratories are significant, and that those owed to the antibiotics are highly significant, which is normal as the resistance of the reference strain varies with each agent. The interaction laboratory-antibiotic is also very significant; this confirms the fact that the differences observed between the laboratories vary according to the antibacterial agents tested.

A one-way analysis of variance undertaken with 15 antibiotics tested by all laboratories confirms that differences between laboratory results are not significant for 7 antibiotics, but are significant to the level $p=0.01$ for the 8 other antibiotics.

Influence of the antibiogram system

In France, there are two antibiogram systems, commercialised by two Institutes designated here by P1 and P2.

The results presented in this analysis have been obtained with the P1 system which is the most frequently used. As each laboratory only uses either P1 or P2 methods, it is therefore not possible to include this variable within the variability analysis.

The global comparison of the average results shows that P1 gives bigger diameters than P2, and with a better precision (smaller standard deviation).

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DISCUSSION

This analysis is just the summary of a more important activity which unfortunately cannot be duely developed here.

The measurements of the antibiotics sensitivity by the discs method is submitted to many other sources of variability. Some are bacteriological, not possible to be measured and linked to the method. Others depend more particularly from the technician in charge of the antibiogram. Among the more important, one must mention the method of measurement of the diameter of the inhibition zone. The producing Institute recommends to use a slide calipers placed as near as possible from the agar medium; actually, these measures are very often made with a graduated ruler placed at the bottom of the Petri dish, the thickness of which introduces an inevitable error due to parallax .

The statistical analysis shows that the variability between laboratories is different according to the antibiotics. It does not automatically means this is a technical insufficiency, but rather a difficulty of measuring depending from the antibiotics with which the inhibition zone is not exactly delimited at the agar surface but is surrounded by an intermediary and woolly border which prevents the reading of the measure. In this case, the above-mentioned phenomenon of parallax can only amplify the error.

CONCLUSION

This statistical analysis of the results of antibiograms realised on a reference strain of E. Coli, within the French network of surveillance of the antibioresistance of pathogenic germs in bovines shows an important variability within the method linked essentially to the laboratories which use it and to the technics they use.

So as to make sure that this measurement procedure remains the best to compare the results between laboratories or to follow-up the evolution of every species sensitivity, it is of the utmost importance that the technical procedures be strictly followed and that the indications provided by the disc producers be scrupulously respected. A permanent quality control survey like the one presented here is not only indispensible but it should also be coupled to a regular revision of the technics used by the different partners, so as to maintain a perfect harmony and to avoid an insidious and inevitable deviationm, inherent to any technics used routinely.

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LAB	AMP	AMO	AUG	APR	ERY	SPI	NOV	POL	COL	TOP	TSU	FUR	NAL	FLU
1	17.7	21.3	18.4	22.2	8.6	9.3	8.4	17.1	18.1	22.9	18.2	17.9	25.1	31.7
2	16.0	20.3	17.6	23.6	9.8	10.0		16.9	18.5	21.5	18.4	16.7	25.2	30.
3	14.5	20.5	18.3	24.1	6.9	8.0	6.0		18.3	28.0	19.4	15.9	26.0	31.
4		23.0	16.5	23.0	6.0	8.5		17.0	18.8	24.5	18.0	20.0	28.0	33.0
5	20.0	22.9	19.0	23.1	7.0	9.0		18.0	19.1		18.6	18.3	26.4	31.9
6	17.0	25.0	20.2	24.0	9.8	8.0		18.0	19.3	19.7	19.5	17.3	26.6	32.3
7	19.0	23.0	18.1	24.6	9.0	8.1		17.0	18.8	23.8	19.0	17.7	23.4	31.1
8	18.5	20.0	17.8	22.8	10.0	6.0	9.5	17.5	18.3	20.0	16.8	14.0	25.5	31.5
9	14.0	20.8	18.6	22.4	6.8	8.0	9.0	18.0	18.9	20.0	18.4	17.0	26.7	32.2
10	19.9	24.0	21.0	24.3	10.9	8.0	12.0	16.8	17.6	23.0	19.0	15.9	27.0	33.0
11	17.7	20.5	16.7	23.1	7.4	8.3	8.0	16.8	18.8	23.0	18.3	17.6	26.7	31.5
12	18.3	21.5	20.5	23.7	7.0	8.5	9.0	18.5	18.3		18.1	18.6	27.9	31.3
13	17.1	22.0	18.9	23.3	8.9	6.7	9.0	17.6	18.8	22.1	18.1	17.1	25.7	30.5
14	16.0	21.5	17.8	24.5	10.8	8.0			18.8	22.9	19.3	16.7	25.1	31.8
15	20.0	20.0	18.7	22.6	6.4	6.0		17.0	18.4	22.6	18.2	18.0	26.7	31.6
16	16.2	20.0	19.2	24.6	13.6	13.0	8.2	17.9	19.4	22.6	19.4	18.0	25.0	30.1
17	19.3	21.4	18.8	23.2	6.6	6.6	11.0	17.0	18.6	22.5	17.7	18.0	26.1	30.5
18	16.7	20.6	17.1	23.3	6.9	7.0	7.0	17.0	18.2	22.1	17.6	17.8	26.8	34.0
19	17.1	22.5	18.4	23.6	7.7	9.8	9.0	18.5	19.2	23.6	18.5	17.6	25.9	31.2
20	16.0	21.9	19.2	23.3	7.3	6.7		16.5	19.1	23.5	17.4	18.3	27.3	31.1
21	16.5	21.0	21.2	26.2	12.0		13.0		20.8		20.0	17.3	26.2	30.0
22	18.4	21.8	20.0	24.5	14.0	6.0			20.9	22.4	19.4	14.6	24.7	30.8

TABLE I. Mean inhibition diameters (in millimeters) obtained in 22 laboratories with 14 antibiotics

Source of variation	Sum of squares	DF	Mean square	F test
Main effects	8124.39	13	624.95	211.71
Laboratory	58.18	9	6.46	2.19
Antibiotic	8066.22	4	2016.55	683.11
Interaction	239.22	36	6.65	2.25
Residual	590.40	200	2.95	
Total	8954.01	249		

TABLE II. Variance analysis

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