THE BACTERIOLOGICAL CONTROL OF SHELLFISH.

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No hygienist has any doubt that diseases of the intestinal tract, especially the enteric fevers, may be spread by the consumption of shellfish. Clinical experience, often repeated, led to this conclusion long before the bacteriologist had been able to detect the presence of the causative organism in the shellfish. In fact it is only within the past year that definite proof has been afforded by the exceedingly valuable work of Wilson (1928), who found *B. typhosus* in cockles taken from Belfast Lough.

The chief reasons for the danger in eating shellfish are:

(*a*) Their preference for a locality where the density of the sea water has been reduced by admixture with fresh river water.

(*b*) The presence of sewage in almost all our rivers.

(*c*) The addition by enteric cases and carriers of the causal organism to such sewage.

(*d*) The fact that shellfish are frequently eaten uncooked.

Wilson and Blair (1927), have demonstrated a practicable method of isolating the typhoid bacillus from water, sewage or shellfish, but there is no reason to believe that their method will replace entirely the methods previously in vogue for the examination of such material. The bacteriologist condemns either a water supply or a sample of shellfish in which he finds definite evidence of faecal contamination despite the fact that the most exhaustive search, using the best methods, fails to reveal the presence of bacilli of the enteric group. The sanitarian confirms the condemnation when an investigation of local topography shows that the contamination is of human origin. The criteria of the bacteriologist are the presence, in what experience has shown to be excessive numbers, of the so-called indicator organisms—*B. coli*, faecal streptococci and *B. welchii*—organisms invariably present in faeces. The water or shellfish are condemned not because the *B. coli* and other bacteria are harmful, almost certainly they are not, but because, where they are found, typhoid and paratyphoid bacilli will also, sooner or later, be present. The bacteriological examination of shellfish is, therefore, chiefly directed towards the finding of the indicator organisms, especially *B. coli*, and an estimation of the numbers in which they are present.

There are two chief methods used in these investigations, of which only the outlines need be stated here. In the first method, that of Houston (cited by Eyre, 1924), 10 oysters are opened and the shell fluid, together with the minced flesh is transferred to a sterile 1000 c.c. cylinder. Sterile saline is

added to make 1000 c.c. and the contents thoroughly mixed. The number of *B. coli* in the cylinder fluid is ascertained by the usual cultural methods and from this the number per oyster is deduced, since 100 c.c. of cylinder fluid represent one oyster. Houston's standards for oysters are: strict standard 100 *B. coli* per oyster; lenient standard 1000 per oyster.

The other method, that of Klein (cited by Eyre, 1924), is to examine a single volume of the liquor and exudate from the minced flesh of a number of shellfish and to determine, for each shellfish, the presence or absence of *B. coli* in the given volume. For oysters the volume used, in Klein's later work, was 0·2 c.c. (0·1 c.c. in the case of mussels) and he concluded that the presence of *B. coli* in this amount corresponded approximately to 200 *B. coli* per shellfish. The method as now used by Prof. Eyre on behalf of the Fishmongers' Company is to examine 10 shellfish of each batch submitted, using 0·2 c.c. with oysters and 0·1 c.c. with mussels of the fluid obtained by mixing the shell liquor with the minced flesh. In the absence of *B. coli* from the given amount of fluid the shellfish is considered "clean"; if *B. coli* is present it is classed as "not clean." The results are expressed as percentages—"20 per cent. clean" or "90 per cent. clean." This method was considered and approved, in 1924, by a committee consisting of Prof. Sir Frederick Andrewes, Prof. Hewlett and Prof. Eyre (1924). The administrative action taken by the Fishmongers' Company, which is based on the results obtained by the method, is as follows. Where 60 per cent. or more of the shellfish are clean the batch is allowed to be sold. Where 50 per cent. or 40 per cent. are clean the batch is held pending further investigations. Where 30 per cent. or less are clean the batch is condemned. It is only fair to state, however, that so far as the Company is concerned, it does not permit the sale in London of shellfish from any locality or bed until a series of chance samplings, such as those referred to, have shown consistently good results, substantiated by topographical evidence as to the suitability of the bed for the growth of shellfish for human consumption. Subsequently chance samplings are compared with those previously recorded and the occurrence of bad results is regarded as evidence of some untoward happening and prompts further enquiry. A single bad result does not condemn an approved bed nor does a single good result permit the sale of shellfish from a suspected locality.

The author's personal experience is chiefly with mussels and the method used by him is fundamentally that of Klein. During the past three years a number of samples have been examined in parallel by the author, acting for the Department of Fisheries of the Irish Free State, and by Prof. Eyre, on behalf of the Fishmongers' Company. Efforts were made to divide the mussels, which were collected and forwarded by Mr Farran, one of the Department's Inspectors, into two identical samples. Table I shows the results of the examinations. In each case the sample consisted of 10 mussels and the results shown are the number out of 10 which were found to contain *B. coli* in 0·1 c.c. of the fluid obtained by mixing the shell liquor with the body juices.

Table I. *Number of mussels considered "not clean" out of* 10 *in each sample.*

Test No.	Prof. Eyre	The author	Test No.	Prof. Eyre	The author
1	4	10	21	10	8
2	10	9	22	3	2
3	2	5	23	9	7
4	9	7	24	1	3
5	3	2	25	9	10
6	2	9	26	7	10
7	10	6	27	10	9
8	6	2	28	9	10
9	7	4	29	4	2
10	9	10	30	0	6
11	1	2	31	0	1
12	1	1	32	0	2
13	1	3	33	1	2
14	3	2	34	1	3
15	1	2	35	2	0
16	1	5	36	3	2
17	1	4	37	3	4
18	8	4	38	6	3
19	7	5	39	3	8
20	6	5	40	5	2

It will be noted in examining the table that there are some irregularities in the results and it is with these irregularities that this paper is chiefly concerned.

The results may first be considered in view of the administrative action which would ensue.

Table II.

Agreements.

Both pass	17	=27 (67 %)
Both hold	1	
Both condemn	9	

Disagreements.

Prof. Eyre	The author		
Passes	Condemns	3	=13 (33 %)
Passes	Holds	3	
Holds	Passes	3	
Condemns	Passes	2	
Condemns	Holds	2	

If we regard the "hold" as an intermediate zone and consider disagreements only as those cases in which one examiner recommends passing the sample and the other rejecting it, we find:

Prof. Eyre	The author		
Passes	Condemns	3	=5 (12½ %)
Condemns	Passes	2	

Ignoring the administrative aspect we can fix an arbitrary number and decide that if the difference between the two results exceeds this figure the tests will be regarded as disagreeing. It seems reasonable to permit as a maximum not more than a 30 per cent. divergence, that is the two results should not differ by more than 3. On this standard there are 8 disagreements (20 per cent.).

The more serious disagreements may now be isolated from the cases in which agreement is reasonably good.

Table III. *Mussels condemned out of* 10 *examined.*

Batch No.	Prof. Eyre's result	Author's result	Disagreement: One passes, the other condemns	Disagreement: Difference of more than 3 per sample
1	4	10	+	+
6	2	9	+	+
7	10	6	−	+
8	6	2	−	+
9	7	4	+	−
16	1	5	−	+
18	8	4	+	+
30	0	6	−	+
39	3	8	+	+

We find that in four cases (10 per cent.) the results are in disagreement when considered on both bases. The percentage of disagreements is therefore possibly as high as 20 per cent., but certainly not less than 10 per cent.

The possible causes of the disagreement are:

1. Errors in labelling samples.
2. Errors in technique.
3. Alteration in the mussels due to delay or conditions of transmission to the laboratories.
4. Errors of sampling.

Since every care was taken, the first possibility does not require further consideration. As regards the second it is only right to point out that Prof. Eyre has had a much longer and far greater experience of examining shellfish than has the author. The technique, however, is not a difficult one and it does not appear likely that this can be a serious cause of error.

At first the third possible cause may appear important. The mussels for the two laboratories were carefully packed but those coming to the author's laboratory were usually brought in by the Inspector and examined within 24 or at most 48 hours of collection. Those for Prof. Eyre were sent by post or rail and, in some cases, five days elapsed before they were examined. So far as I can ascertain a delay of even this duration, under reasonable conditions, does not cause any great alteration in the bacteriological condition of the shellfish.

The fourth possibility, which is believed to be of far the greatest importance, is that the disagreements are almost entirely due to chance differences in the mussels examined. When the results are added together it is found that Prof. Eyre classes 178 as "not clean" and the author puts 191 in this class out of the 400 mussels examined. These figures correspond to 4·45 and 4·78 for the usual batch of 10 mussels, a disagreement of just over 3 per cent., which points to a high degree of agreement when the number of mussels is large and which renders improbable any of the first three suggested causes of disagreement.

In considering the question of sampling errors it was thought that Poisson's formula might be applicable but Prof. J. L. Synge states that the number in the samples was far too small to use this formula. By a special consideration of the problem Prof. Synge arrived at the conclusion that, in certain cases, either the sampling or testing was unsound.

Various trials made by drawing samples of beads, identical except for colour, from a large number containing different proportions of the two varieties confirm the opinion that the differences are largely due to chance, but it was thought advisable to make a test on mussels. Mr Farran supplied a large number of mussels from the River Nanny from which four samples, each of 10 mussels, were taken at random and examined. The numbers found "not clean" were 6, 2, 7, 5. The maximum divergence between any two of these results is 5, a number only exceeded on three occasions in the 40 parallel tests. In this case the mussels were examined at the same time by the same bacteriologist using the same technique, and the only possible error is that of sampling.

For the reasons just given the author believes that the differences in the results obtained by Prof. Eyre and himself are almost entirely due to chance errors of sampling.

The element of chance in sampling operates in two ways, both of which must be considered:

1. Chance in the selection of the mussels for examination.
2. Chance in the selection of the particular fraction of mussel fluid to be examined.

If we have a very large batch of mussels of which equal numbers are "clean" and "not clean" and a number of samples of 10 are taken, some of these may show 10 "clean," some 10 "not clean" but the majority will approximate to 5 "clean" and 5 "not clean." Where only one or two samples are taken results may be obtained which are very far from representing the true condition of affairs. If "clean" and "not clean" were characteristics absolutely distinct as are "head" and "tail" in tossing coins, our only way of obtaining reliable results would be to increase the size of our sample to say 100, a quite impracticable proposition for routine work. The Committee already referred to, in 1924, recommended the examination of 10 shellfish (instead of 6 as previously used) in each sample. "This will help to diminish any possible element of chance in the number found contaminated." The increase undoubtedly helps but it is insufficient to rule out this source of error. Further, in the case of mussels, "clean" and "not clean" are relative terms. A mussel with only 10 *B. coli* is certainly "clean" and one with 1000 is "not clean," but can we so differentiate between one with 199 *B. coli* and one with 201 *B. coli*? Mussels taken from the same bed do not, for the most part, show wide variations in bacterial content. One may have ingested recently a relatively large unresolved faecal particle and so show very much larger numbers of *B. coli* than the majority, but this is exceptional. Given impossibly perfect

bacteriological technique and practice agreeing with mathematical theory, an observer, using Klein's method, might class as 100 per cent. "clean" mussels containing 190 *B. coli* each and as 100 per cent. "not clean" mussels containing 210 *B. coli* each, and yet for all practical purposes the two samples are identical. The great disadvantage of Klein's method is that it is a "hit or miss" method; the results must come out "*B. coli* present" or "*B. coli* absent" and so the mussel must be classified as "not clean" or "clean." We cannot regard as entirely satisfactory any method which does not throw light on the degree of "cleanness" or "not cleanness." The method introduces chance a second time, in the selection of the particular fraction of fluid for examination. We know that if we have a fluid containing exactly 100 bacteria per c.c. and we take a number of volumes of 1/100 c.c. some will contain no bacteria, some one and some two or more. If, however, we take samples of 1/10 c.c. practically all will show the presence of bacteria and with samples of 1/1000 c.c. practically none will show their presence. By taking the three volumes, 1/10, 1/100, 1/1000 c.c. we are given a much truer idea of the bacterial content of the fluid than by taking only 1/100 c.c.

Can these two operations of chance be controlled in any way? As regards the first, the chance in the selection of the mussels, the obvious way, by increasing the number in each sample to 100, has been ruled out as impracticable. But since, in the majority of cases, the variation of individuals from the mean for the bed is inconsiderable, a method which will indicate more precisely the number of *B. coli* present in place of fixing them at more or less than 200 will, very largely, remove this source of disagreement. Such a method will also go a long way to remove the second source of error, that due to the operation of chance in the selection of fractional volumes from the mussel fluid. Some bacteriologists engaged in the examination of shellfish use Houston's method, but this does not commend itself to the author chiefly because one bad mussel in the sample will lead to the condemnation of the entire sample and there are very many ways in which one bad shellfish might be introduced into an otherwise entirely satisfactory batch. If we have 9 oysters, each containing 40 *B. coli* and 1 with 10,000, the average number per oyster will be over 1000 and, if the mixture has been satisfactory, the entire batch will be condemned as exceeding even Houston's lenient standard. Were an exactly similar batch examined by Klein's method not more than 3 or 4 oysters should be found "not clean" and the batch would pass.

For some years past the author has used a method which, while it increases considerably the use of media, does not greatly increase the time devoted to the examination but does give additional information of considerable value.

0·5 c.c of the mixed fluid from the mussel is added to 4·5 c.c. of sterile saline, giving a 1 : 10 dilution. Of this 2·0 c.c. are added to one tube of lactose bile broth, 1·0 c.c. to a second and 0·2 c.c. to a third. The second tube corresponds to the one used in the ordinary Klein method and a positive result corresponds to 200 or more *B. coli* per shellfish. A positive result in

the first corresponds to 100 *B. coli* and in the third to 1000 *B. coli* per mussel respectively. We have then three tubes for each mussel, supplying a strict, moderate and lenient standard. The strict and lenient standards are the same as those of Houston, the moderate one being the same as Klein's standard. The method minimises the errors due to the operation of chance both in sampling mussels and in sampling the contents of a mussel, it shows whether the contamination is very heavy, moderate or slight, and it does not permit 1 bad shellfish to outweigh the goodness of the other 9 in a sample.

Some examples from my records will make the advantages clearer. In each case the percentage of "not clean" mussels is stated under the three standards, strict, moderate and lenient.

Table IV.

A	10	10	0
B	50	10	0
C	30	10	10

All these pass but sample *A* is best. *B* shows a considerable percentage with *B. coli* approaching the limiting figure, and *C* a small percentage with a high degree of contamination.

Table V.

A	20	20	0
B	60	20	10

Both pass, but in *B* more than half fail to pass the strict standard while 10 per cent. fail in the lenient standard.

Table VI.

A	40	30	0	*D*	80	30	20
B	40	30	10	*E*	100	30	20
C	50	30	10				

These five samples are all allowed to pass with 30 per cent. "not clean" by Klein's method, but when the results of the other tests are examined they are found to form a series showing gradually increasing degrees of contamination.

Table VII.

A	30	40	0	*E*	70	40	0
B	40	40	20	*F*	70	40	20
C	50	40	10	*G*	70	40	30
D	60	40	10	*H*	80	40	20

Despite the identical results obtained by Klein's method, which allows all these samples to pass, the series shows great diversity of results when judged by the other standards. *A* is an example of an error of sampling the fluid of a mussel and would be expected to appear at 30 per cent. "not clean" instead of 40 per cent. in the moderate standard. *B* with 20 per cent. of failures to pass the lenient standard must be considered worse than *D* despite the fact that the failure on the strict standard is greater in the case of *D*. In general, failures on the lenient standard are more serious than on the strict.

E, *F* and *G*, with exactly the same results on the strict and moderate standards, are differentiated by the increased percentage failing to pass the lenient standard.

Table VIII.

A	50	50	10	*E*	70	50	20
B	60	50	0	*F*	70	50	30
C	60	50	20	*G*	80	50	30
D	60	50	30	*H*	100	50	20

These are examples of the intermediate group to be held for further consideration. The additional results in the strict and lenient standards should certainly be of assistance in interpreting the results. The superiority of *A* and *B* over *G* and *H* is obvious.

Table IX.

A	70	60	10
B	60	60	50
C	100	60	60

Again the further knowledge afforded by the additional standards in the case of mussels held for further consideration is demonstrated.

Table X.

A	90	70	20	*G*	100	90	50
B	90	70	30	*H*	100	90	80
C	100	70	70	*I*	100	100	50
D	80	80	40	*J*	100	100	80
E	90	80	60	*K*	100	100	90
F	100	90	40	*L*	100	100	100

This illustrates the varying degrees of badness in mussels which are to be condemned.

Sufficient has, it is hoped, now been said to prove that the modification of Klein's method here suggested is one which does not render the method too expensive in media or too time-consuming to be adopted and which gives further useful information regarding the bacterial content of mussels or other shellfish. It removes some of the objections urged against Klein's method and renders the latter definitely superior to the method of Houston.

The chief criticism which may be made against the proposed method is the difficulty in giving due weight to the three standards, but this is easily obviated by the use of the "weighted mean." It is easily understood that failures to pass the moderate standard are more serious than in the case of the strict standard and failures to pass the lenient standard still more serious. Mathematically the proportion should be 1 : 2 : 10 but to adopt this proportion would place too much emphasis on the last standard, the lenient one. It is therefore suggested that the proportion be fixed at 1 : 2 : 7. The "weighted mean" is found by adding together the number of mussels found "not clean" on the strict standard, twice the number on the moderate standard and seven times the number on the lenient standard. The cleanest mussels will have a weighted mean of 0, the dirtiest 100 and the result can therefore be expressed as a percentage of contamination. It is suggested that a result

not greater than 30 should allow the batch to pass, that between 31 and 40 should hold up the batch for investigation and that a result exceeding 41 should condemn.

Table XI shows some actual results; the weighted mean, the action to be taken according to the rules of the Fishmongers' Company and the action suggested by the new standards.

Table XI.

Example	Number "not clean" (author's method) Strict	Moderate	Lenient	Weighted mean	Action to be taken on new standard	Number "not clean" (Klein's method)	Action to be taken on Fishmongers' Company's standard
1	3	1	1	12	Pass	1	Pass
2	2	2	0	6	Pass	2	Pass
3	4	3	0	10	Pass	3	Pass
4	8	3	2	28	Pass	3	Pass
5	10	3	2	30	Pass	3	Pass
6	3	4	0	11	Pass	4	Pass
7	5	4	1	20	Pass	4	Pass
8	7	4	2	29	Pass	4	Pass
9	7	4	3	36	Hold	4	Pass
10	5	5	1	22	Pass	5	Hold
11	6	5	3	37	Hold	5	Hold
12	8	5	4	46	Condemn	5	Hold
13	7	6	1	26	Pass	6	Hold
14	10	6	6	64	Condemn	6	Hold
15	9	7	2	37	Hold	7	Condemn
16	9	8	6	67	Condemn	8	Condemn
17	10	10	9	93	Condemn	10	Condemn

It only remains to emphasise the various disadvantages inherent in the methods now used in the bacteriological examination of shellfish and the discordant results which occur and to urge those interested in the question to give the method here suggested a trial.

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