J. Hyg., Camb. (1976), **76**, 91
Printed in Great Britain

Observations on environmental contamination in a microbiological laboratory

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(*Received* 20 *May* 1975)

SUMMARY

Contamination of a laboratory environment with pathogenic or non-pathogenic micro-organisms may be relevant to safety of technicians and quality of technical performance. Two widely separated incidents in 1968 and 1974 initiated a study of aspects of the laboratory environment. Water-baths, water of syneresis and portions of salmonella cultures spurting out of the sterilizing flame were examined. The water of water-baths was shown to be contaminated from the fluid cultures incubated in them. This raised questions of potential cross-contamination and reporting of false positives. Water of syneresis was sometimes contaminated with salmonellas. A few quantitative counts were made. The range of counts varied between 16 salmonellas per ml. and 13,000,000 salmonellas per ml. Five hundred portions of salmonella cultures and 571 portions of *Shigella sonnei* cultures which had spurted from the sterilizing flame were examined. All these samples failed to grow salmonellas or shigellas.

Precautions necessary to avoid environmental contamination are briefly discussed.

INTRODUCTION

Environmental contamination with pathogenic micro-organisms is important in the epidemiology of certain diseases in animals and man. Contamination of the environment of a microbiological laboratory with pathogenic or non-pathogenic bacteria is also significant. Here, safety of technical personnel and quality of technical performance are involved. Two widely separated incidents in 1968 and 1974 initiated a selective study of parts of the laboratory environment. During an inter-laboratory quality-control exercise designed to test efficiency of salmonella isolation, many false positive results were reported. Artificially contaminated dried egg powder was the material circulated to participating laboratories. In Cardiff, at that time, water-baths set at 43° C. were used for incubating salmonella enrichment broths, as temperature control was more accurate than with dry incubators. During the course of the exercise, the water in the water-bath was found positive for salmonellas of the same type as was present in the dried egg samples. Cross-contamination of negative specimens from positives was considered possible. The second incident involved bacteriological testing of steam sterilization efficiency in the University Hospital of Wales (Joynson, 1975). Dried

spores of *B. stearothermophilus* (Oxoid code number BR23) were placed near the centre and bottom of a load being sterilized in an autoclave. These spores survive at 121° C. for 5 min. but are killed at a temperature of 121° C. for more than 15 min. After completion of the autoclave cycle, the spore preparation was removed and added to 10 ml. of Oxoid tryptone soya broth in a screw-capped universal container with aseptic precautions. The spores and medium were incubated in a 56° C. water-bath for 4 days. Growth indicated that the spores were viable on transfer to the culture medium. A control fluid culture medium in a similar universal container which had not been exposed to heat was included with each batch of tests. In February 1974 a series of positive spore tests occurred without adequate explanation. As was the case with the salmonella incident, attention was directed to the water-bath as a possible source of contamination. Certain other areas of the environment also seemed worth investigating and from these we drew our materials. A brief description of the salmonella study has been published elsewhere (Harvey & Price, 1975).

MATERIALS

Three materials were examined:

(1) Water was taken from water-baths used to incubate salmonella enrichment broths and tryptone soya broths. The former was set at 43° C., the latter at 56° C. Samples were removed from the 43° C. bath at 24, 48 and 72 hr. from the start of incubating the salmonella enrichment broths. Six separate 100 ml. quantities were taken at each sampling time so that a total of 1·8 l. were removed from the bath on each occasion a batch of enrichment broths was being incubated.

The water in the 56° C. bath was examined at the beginning of April 1974 on finding 29% and 14% of spore indicator tests positive in February and March 1974 respectively. The sample volume was 10 ml.

(2) Water of syneresis was the second environmental sample studied. This is fluid which is extruded from agar plates on standing. We find that colonial differentiation on brilliant green MacConkey plates is optimum if they are left for some days at room temperature. Salmonella colonies develop a rugose surface and this aids their isolation. This phenomenon was described in detail by Jamieson (1966). The fluid which collected on the inner surface of the Petri dishes during the period at room temperature was removed and examined for salmonellas.

(3) In our laboratory, colonial differentiation and slide agglutination of salmonella and shigella colonies is concentrated in a relatively small area of bench working space. This is determined by optimum lighting conditions and skill of technical personnel. This bench area is an environment at risk. During the course of slide agglutination, portions of inspissated salmonella and shigella colonies spurt from the platinum loop, when it is sterilized in the bunsen flame. This material was collected at the end of the working day and examined for the appropriate pathogens.

Table 1. *Isolations of salmonellas from water-bath*

No. of 100 ml. samples positive	No. of times found
6	6
5	2
4	1
3	2
2	1
1	3
0	17

METHODS

Methods of demonstrating salmonellas in materials were conventional. Water-bath samples were added to 100 ml. of double-strength selenite F broth and incubated at 43° C. for 24 hr. Subcultures were made to brilliant green MacConkey agar (Harvey, 1956) and plates were incubated at 37° C. for 24 hr., when suspicious colonies were picked for investigation. Water of syneresis was inoculated on brilliant green MacConkey agar for qualitative examination and salmonella counts were performed on nine samples by a surface counting technique (Miles & Misra, 1938). Portions of inspissated colonies were ground in a Griffiths tube, inoculated into peptone water and incubated at 37° C. for 24 hr. Subcultures were made on brilliant green MacConkey agar and deoxycholate citrate agar. The peptone water was checked for its ability to allow multiplication of minimal numbers of salmonellas and shigellas – the normal quality control test for any fluid medium.

The samples from the 56° C. water-bath were examined by a technique designed to encourage isolation of *B. stearothermophilus*. Ten ml. of Oxoid tryptone soya broth was inoculated with 0·5 ml. of the water sample and incubated at 56° C. for 4 days. Subcultures were then made on blood agar plates and the identity of the organism isolated was confirmed morphologically, culturally and biochemically.

RESULTS

Salmonella isolations from the 43° C. water-bath are given in Tables 1 and 2 in terms of the number of 100 ml. volumes found positive and the time of examination. The majority of isolations were made at the 48 hr. sampling time.

Qualitative and quantitative findings in the water of syneresis study are recorded in Table 3. One hundred and thirty-six samples were examined, of which 29 were positive. Estimates of the numbers present per ml. are given for nine tests. The range of counts obtained was 16 salmonellas/ml.–13,000,000 salmonellas/ml.

Five hundred samples of inspissated salmonella cultures and 571 similar samples of *Shigella sonnei* cultures collected from the bench were examined. Not one specimen yielded viable salmonellas or shigellas on culture.

The examination of the 56° C. water-bath is essentially a separate study, but the results complement the isolations of salmonella species from the 43° C. water-bath. The record of spore indicator tests over a 13-month period is relevant and is

Table 2. *Timing of positive findings*

Duration of enrichment broth samples in water-bath (hr.)	No. of bath-water samples positive at different times
24	11
48	45
72	0

Table 3. *Quantitative and qualitative results in water of syneresis study*

Sample no.*	Salmonella counts per ml.
1	5,500
2	16
3	35,000
4	500,000
5	250
6	525,000
7	13,000,000
8	26,000
9	850,000
Total samples examined	136
Total samples containing salmonellas	29

* The volumes of samples of water of synthesis varied from 0·1 to 0·5 ml.

given in Table 4. At the beginning of April 1974 water in the bath was cultured after the occurrence of an unusual and unexplained number of positive spore tests in February and March. *B. stearothermophilus* was found in three samples. Dry-air incubation replaced water-bath incubation at the end of April. Since the change, only one positive spore test has occurred over a period of 8 months.

DISCUSSION

Isolation of salmonellas and *B. stearothermophilus* from the 43 and 56° C. water-baths respectively is important. If organisms can leak from containers into the surrounding water, it is possible that they can pass in the reverse direction and enter containers previously free from contamination. Reporting of false positive results is, therefore, a danger and is known to have happened in 1968 with salmonellas and probably also occurred in 1974 with *B. stearothermophilus*. The incidents emphasize the unsatisfactory seal between screw-caps and the containers they are intended to close. Contaminated water should never come into contact with unsealed or imperfectly sealed vessels which contain material capable of supporting bacterial growth. This has been clearly demonstrated in certain outbreaks of enteric fever associated with pasteurized milk and tinned meat (Thomas, Stephens, King & Thomson, 1948; Howie, 1968).

Table 2 shows that most positive specimens from the 43° C. water-bath were obtained at the 48 hr. sampling time. This throws light on the mechanism of leakage. The sequence of events was to remove containers from the bath for the 24 hr. subculture and follow this with sampling the bath water. On removal,

Table 4. *Spore indicator tests: December* 1973 *to December* 1974

1973	Spore test result: Negative	Positive	Positive as integer (%)	
Dec.	73	0	0	
1974				
Jan.	74	0	0	56° C. water-bath used
Feb.	37	15	29	
Mar.	66	11	14	
April	71	0	0	
May	50	0	0	
June	53	0	0	
July	74	1	1	
Aug.	62	0	0	56° C. dry incubator used
Sept.	64	0	0	
Oct.	69	0	0	
Nov.	49	0	0	
Dec.	63	0	0	

containers were gently rotated to mix the contents, lids were removed and subcultures made. Enrichment broths must have come into contact with the ill-fitting lids during these manipulations and leakages over the rims of the jars must have occurred. When jars were replaced in the bath for further incubation, salmonellas must have been already transferred to the outside surface in a thin film of nutrient enrichment medium. Positive samples of bath water taken 24 hr. later probably reflected leakages which took place when containers were opened for the first time. Jars were not replaced in the bath after the 48 hr. subculture and so the bath water was not re-contaminated. The water at 43° C. was a hostile environment without adequate nutriment and at the 72 hr. sampling time no salmonellas were found.

This explanation is, of course, hypothetical. We attempted in 1968 to cross-contaminate sterile enrichment broths by incubating them in the same water-bath as salmonella-contaminated material. The experiment failed, but the danger is real and the evidence of an unexplained number of positive spore tests recorded in February and March 1974 and their virtual cessation when dry incubation replaced water-bath incubation is very suggestive (Table 4). It is probable that the 56° C. water-bath was contaminated from the control culture bottle containing viable *B. stearothermophilus*. The bacillus survived in its new environment and subsequently re-contaminated culture bottles containing the test spores to produce spurious results.

Counts on the water of syneresis covered a wide range (Table 3). It did not matter whether the agar-filled portion of the Petri dish was downward or upward. Positive results were still obtained. The empty part of the Petri dish was on top in sample six when a salmonella count of 525,000/ml. was obtained. In sample seven the count was 13,000,000/ml. and the number of organisms which could have contaminated fingers in careless handling was of the order of an infecting

https://doi.org/10.1017/S002217240005498X Published online by Cambridge University Press

dose. This dose varies widely and depends partly on serotype (McCullough & Eisele, 1951*a*,*b*).

Portions of cultures which spurt from the flame during sterilization are noted as a potential hazard with mycobacteria (Collins, 1974). They might also be relevant with *S. typhi*, where the infecting dose for man is small and where there is evidence of a danger of laboratory infection (Sulkin & Pike, 1951).

The precautions necessary to prevent contamination of the environment are evident. Dry incubators should replace water-baths unless temperature regulation is absolutely critical. Care in agitation of screw-capped containers should be observed. Leaving selective agar plates at room temperature should be kept to a minimum and should be reserved for those samples in the examination of which it is advantageous. The use of hooded bunsens should be considered when flaming platinum loops and protective inoculation of technicians should be used when material contaminated with *S. typhi* is handled. Protective inoculation with T.A.B. vaccine will, of course, be valueless against members of the salmonella group other than the enteric organisms.

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