METHOD EMPLOYED AT NEW YORK QUARANTINE FOR THE DETECTION OF CHOLERA CARRIERS.

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In July, 1911, the cholera epidemic in Italy reached such proportions, and so many cases of cholera developed among Italian immigrants en route to the United States and among those detained after arrival in quarantine at New York and other ports on the Atlantic seaboard, that quarantine regulations of the Federal Government relating to cholera were amended so that all steerage passengers coming from cholera infected ports to this country should be held for bacteriological examination before being discharged from quarantine. The new regulations were promulgated in order that cholera carriers might be detected and detained. Accordingly, all ships from cholera infected ports were held at quarantine and fecal specimens were taken from all the steerage passengers and examined for the presence of cholera vibrios.

At the New York state quarantine station, from the middle of July to the middle of November, 1911, the number of immigrants examined was 26,930, of which the New York City Laboratory examined 3,900. On some days the number of examinations did not exceed 300, but not infrequently it was twice as many, and sometimes reached 1000. On one occasion over 2,000 steerage passengers arrived at one time. Such fluctuation of work entails a strain on the laboratory staff that can not press into service a large corps of emergency workers, and in this category, of course, fall the majority of quarantine laboratories.

As the work progressed, there came a realization that the efficiency of the examination would be promoted by adopting procedures that would expedite the work, and so from time to time, with this object in view, changes in technique were made.

The object in describing the methods used at New York is rather to set forth results accomplished in hastening the work than to present anything essentially new in cholera examination, for the expeditious methods finally evolved may serve as an aid to those who may be confronted with the examination of a large number of cholera suspects, and who may have only a small laboratory force available for the work.

NOTE.—The simplification and abbreviation of methods were due to suggestions made by Dr. C. E. Baldwin, in charge of the laboratory, Dr. W. F. Magill, head of the New York state laboratories, who coöperated in the work throughout; Dr. Serrati, of the Italian Navy, Royal Commissioner at Port of New York; Dr. A. J. Bendick P. A. Surgeon McLaughlin, P. A. Surgeon von Ezdorf, and the writer from United States Public Health and Marine-Hospital Service.

The methods advocated are as follows:

Obtaining specimens: If the passengers have to be held on board ship, rectal swabs are recommended, for without doubt this method is the quickest and is the surest, because it prevents confusion of specimens and because it prevents substitution on the part of those examined. One man with a clerical assistant can take 150 swabs an hour from men, and 100 an hour from women and children. It is best to employ a female operator to take specimens from women. If the professional force is small a skilled nurse can perform this work. If the persons to be examined can be segregated in properly equipped quarters and if an adequate force of assistants is available, swabs from stools obtained by the use of salts are preferable to rectal swabs in working to detect carriers, because a larger amount of material is available for examination.

As each person passes through a room set aside for the purpose of taking swabs, a number is placed on a card bearing the passenger's name, manifest number, and the ship's name, all of which has previously been made out, and the same number is affixed to a peptone tube into which the swab is to be dropped. In taking the specimen, a cotton swab similar to an ordinary throat swab is used, after having been previously dipped into a peptone solution, which serves as a lubricant and maintains the viability of the specimen.

At first the inoculations were made in the laboratory and the swabs were conveyed to and from the ships in individual tubes, but this method was abandoned for direct inoculation of culture tubes at the time of securing the specimen, the swabs being taken to the ship in sterile packages of 100. The change resulted in very material saving of time and labor. By the skill and dexterity which come with experience, the swabs can be passed without a speculum, but individual glass specula were used to some extent. The latter consist of small glass tubes open at both ends, and with rounded edges. They can be inserted with facility and cause less discomfort than passing the swabs direct and their use permits of the removal of a greater amount of the desired specimen. With subsequent sterilization the same tubes can be utilized over again on other suspects.

As the inoculation of the stool was made direct from person to peptone tube it was necessary to take the culture tubes to the ship numbered and ready for inoculation. The tubes were carried in wooden blocks such as are used in laboratories, each block contained forty tubes set upright in holes bored to hold them. Wire test tube racks are superior to the wooden blocks but are not so easily made. It is necessary to number only the end tubes in the blocks.

Subcultures: The original cultures were incubated six hours and then inoculated into fresh peptone tubes. The time element is not so important in the incubation of subcultures; from six to ten hours was the usual period employed by us.

In the latter part of work subcultures were made in a special saccharose peptone medium devised by Dr. Arthur Bendick.* This medium seems to promise the elimination of smears from such cultures as do not contain cholera or cholera-like vibrios.

In making subcultures two radical departures were made from the technique generally used. (a) Cotton plugs were discarded. This is open to two possible objections; first, difficulties may arise from aerial contamination; second, danger may result to the laboratory force through scattering of the infection. As to the first, it is a negligible factor. In several thousand tubes so treated no confusing growth resulted, partly, no doubt, because the peptone solution is a poor medium for many bacteria. The tubes, when prepared, were placed in blocks and each series covered by sterile muslin cloth securely fastened in place. As to dissemination of infection, the only precaution necessary is the complete elimination from the laboratory of flies and other insects. The avoidance of handling plugs saves much time. (b) The second divergence from routine technique consisted in the use of individual wire loops for the transfer of cultures. These loops were made from a light composition wire and sterilized in bundles of 100. By using these loops and by placing the rack containing the original cultures beside the corresponding rack of tubes to be subcultured one man can do three hundred subinoculations an hour. The individual loops save the time consumed in sterilizing an inoculating needle. On transferring a drop from the upper layer of the original culture to the subculture the loop is dropped into the latter. The question arose as to whether the metal, by remaining in the culture medium, exercised any restraining influence on the growth of inoculated organisms, but control experiments failed to show any such effect.

Smears: Smears from subcultures are strongly advocated. There are a number of reasons why the examination should be confined to subcultures in examinations to detect carriers; there are, too, several objections to the use of the original cultures for this purpose. First, reliance on the smears from subcultures prevents useless plating, for not infrequently a smear from an original tube shows forms that prevent the discarding of the culture as negative. These forms appear to be isolated, suspicious-looking, curved rods. They are really artifacts, due to stain, or to detritus of media, or they may be distorted bacilli or non-pathogenic vibrios that

* See page 906.

do not multiply and do not appear in the subculture. This applies only to the original cultures, for however few the cholera vibrios are in the specimen, and however scarce they appear in the smears from the original culture, the smear from the subculture always shows a large number of cholera vibrios in every field. The examination of five fields or less is sufficient when smears are made from subcultures. Secondly, subculturing facilitates isolation of cholera colonies from the plates, for if only a few vibrios are present in a specimen, and this may frequently happen in carrier cases, they may be lost on plating, either through the dilution of the culture incidental to plating, or through overgrowth by other colonies. Special plate media obviate this last possibility, but special plate media in comparison to alkaline agar require too much time and trouble for practical purposes, and plating from subcultures renders them non-essential.

Plating the original culture on agar, with failure to isolate a vibrio afterwards discovered to be present in the culture, probably has given rise to term "non-enrichment." This term as generally used apparently implies a condition of latency. That any cholera organism fresh from the intestines will not multiply greatly when transferred to an optimum medium seems wholly improbable. Neither in cases nor carriers was this observed in the series of sixty-two cases occurring at the station. It is readily understood that, among carriers, if the specimen inoculated contains only a few vibrios, the increase from these after six hours incubation may be so limited as to cause the vibrios to be overlooked in the smear. This actually occurred during last summer's work in five specific cases, two of which were reported by Dr. Krumwiede from the New York City Laboratory. In each instance, though, the smear from the subculture showed at a glance large numbers of vibrios. Also a number of cholera-like vibrios that did not appear in the examination of originals were observed in the smears from subcultures and easily isolated from the latter.

It was our custom to make smears on glass slides, putting five on each slide, and numbering the first and last smear with a grease pencil, the intermediate smears being indicated by a small cross. No trouble whatever arose from the obliteration of the grease pencil marks. The material for the smears was taken from the upper layer of the culture and, as in the method of subinoculation, individual loops were made use of for this purpose. One worker can make 150 smears an hour. Drying of the smears was hastened by passing the slide through a flame until the slide became hot, and then, after the slides had cooled, the smears were stained with dilute carbol-fuchsin—one part of carbol-fuchsin to eight parts of distilled water.

Examination of smears: In consideration of the fact that subcultures always showed the vibrios in great numbers if they were present at all, a prolonged search of the stained smear for vibrios is not necessary. One worker can examine three hundred smears in eight hours.

Plating: In making plates a drop from the upper layer of the subculture is diluted by adding it to a tube of fresh peptone solution, and one drop of the dilution is put on a plate and rubbed in with a glass rod. In subculturing, in making smears, and in plating, care should be exercised to avoid any surface pellicle. In no instance was difficulty experienced in isolating cholera or non-cholera vibrios from subcultures by the use of alkaline agar plates. A fairly large experience shows that, if subcultures are employed, the use of special plate media such as Dieudonne's or the even better alkaline egg medium devised by Dr. Charles Krumwiede*, is an unnecessary refinement of technique. All the advantages that Endo's medium possesses for isolation of *B. typhosus* obtain in cholera work if the alkalinity of this medium is increased to double that usually employed, for on this medium vibrio colonies give a very typical, clear, amethystine color.

Identification of vibrios: The final diagnosis is determined by the isolation of the vibrio in pure culture and its instantaneous agglutination with specific serum in dilution of 1–400, the controls being negative. In one instance I worked with a vibrio similar to the cholera vibrio in morphology, motility and also in cultural characteristics, which agglutinated with specific serum in a dilution of 1–400 after fifteen minutes, and did likewise with normal serum in the same dilution. As the clumping of the organism in specific serum was not observed in any higher dilution than that of normal serum the organism was excluded as a cholera vibrio. Subsequently, for experimental purposes, the same vibrio was swallowed by a laboratory worker without any ill effects.

For provisional diagnosis when a vibrio was found in a smear mixed cultures were tested in the hanging drop. If there was no cessation of motility when the cultures were treated with cholera serum in low dilution a tentative decision was made that the vibrio was not cholera. If there was clumping of the mixed culture in high dilution it was considered positive until the examination of the suspected organisms in pure culture settled the matter. Cultural tests were not relied on to confirm the identity of the cholera vibrio. The cholera-red reaction as applied to mixed cultures was dropped as useless, for many negative cultures gave various shades of red when treated with concentrated sulphuric acid. The motility of the cholera vibrio is characteristic but is not distinctive, for any polar flagellates, including non-cholera vibrios and pseudomonas, have the same rapid darting motion.

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SUMMARY.

In summarizing the preceding paragraphs on methods the following salient points are epitomized:

In obtaining specimens, if it is necessary to detain the suspects aboard ship, or if the staff conducting the examination is small, the use of rectal swabs is advised, for it is a quick method and it prevents deception and substitution of specimens on the part of those undergoing examination. Furthermore, if the procedure has to be confined to ship's quarters, the administration of a saline cathartic carries with it the possibility of disseminating infection. If the passengers can be segregated on shore and a large supervisory force is available, the administration of salts with a subsequent swab from the stool is a preferable method of obtaining specimens.

To save time the use of cotton plugs in the tubes holding both the original culture and subcultures can be discarded, if the proper precautions are observed.

The use of subcultures instead of the original culture in the preparation of smears is urged.

In making subcultures, Bendick's saccharose peptone medium promises material curtailment of work in the detection of cholera vibrios. Special plate media is not necessary in working with subcultures. A two per cent. alkaline agar is entirely satisfactory for plating out vibrios. In plating from cholera cultures, Dr. Krumwiede's special plate medium seems to possess several advantages that Dieudonne's medium does not.

In making subcultures and smears, individual loops save the time consumed in flaming the platinum loop ordinarily used for this work.

Non-enrichment, in so far as it implies latency, was not evident in cultures from sixty-two carriers and cases.

EFFECTIVENESS OF BACTERIOLOGICAL EXAMINATIONS IN QUARANTINE ADMINISTRATION.

By means of bacteriological examination, twenty-seven cholera carriers were discovered in passengers arriving at and in detention at the New York Quarantine station. On one ship which had two carriers there had been no recognized cases en route.

Because of the regulations of the Federal Government pertaining to immigrants from cholera infected ports, and because it was presumably imbued with the desire to coöperate, the Italian Government started examinations for carrier cases early in the summer of 1911. As a result forty carriers were discovered in Italy among prospective immigrants.

Following the inauguration of bacteriological examinations by the Italian Government there ensued a subsidence of carriers and cases at the New York Quarantine Station. This decline was no doubt partly due to the fact that in the latter part of the summer immigrants were detained five days at the port of departure before sailing. Thus is emphasized the necessity of thorough precautions at the point where quarantine measures should be most rigorously enforced, namely at the port of departure.

Bacteriological examinations not only confer the maximum amount of protection to a non-infected country but they also work the least hardship on commerce and the traveling public. In lieu of the expensive, irksome detention for an uncertain period at quarantine, bacteriological examinations allow the release of the ship and a large majority of her passengers after a delay of only 24 to 48 hours, according to the number of passengers, and that, too, with greater safety than was attained by the old clinical standard of quarantine.

Examination of contacts can be made within 48 hours after the separation from case or carrier. Any ingested vibrios being subject to the same conditions of mechanical transmission as the rest of intestinal contents, regardless of symptoms, will probably appear in the stool within 48 hours. This assumption is supported by the results of an experiment in the laboratory; two of the bacteriologists swallowed a fresh culture of a cholera-like vibrio and recovered vibrios from a stool within thirty hours.

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