PROCEEDINGS OF THE PLAGUE COMMISSIONER.

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MISCELLANEOUS.

No. 272

Dated 27-1-99

Despatched

[Despatch Abstract.]

M. Haffkine's Serum

Recording copy of report of Capt. C. A. Johnston to the President, Indian Plague Comm. on the purity of ——————.

BACK PAPERS.

No.	Date.	No.	Date.

FURTHER PAPERS.

No.	Date.	No.	Date.

Plague—Hyderabad State.

FROM

CAPTAIN C. A. JOHNSTON, M. B., D. P. H., I. M. S.,

Medical Officer, 3rd Madras Lancers.

To

THE PRESIDENT,

Indian Plague Commission.

Secunderabad, 9th January 1898.

SIR,

I have the honor to forward herewith as requested by the Indian Plague Commission a report on a bacteriological investigation into the purity, or otherwise, of Haffkine's plague prevention inoculation fluid, accompanied with stained preparations of some of the organisms found.

The report has had to be drawn up rather prematurely this day as I have to accompany my regiment out of Secunderabad on "Long reconnaissance," and shall be absent from the station for a week. I therefore regret that it cannot be so fully drawn up as it otherwise might have been.

I have the honor to be,

Sir,

Your most Obedient Servant,

(sd) Chas. A. Johnston

M. B., D. P. H., I. M. S.,

Medical Officer, 3rd Madras Lancers.

Bacteriological Examination of Haffkine's Fluid.

Seven bottles of Haffkine's fluid with the following numbers, taken at random from about 50 others, were examined :—

Brew No.	4168 (A)	received at Hyderabad on	19th November 1898.
,, ,,	5324	do. do.	16th December 1898.
,, ,,	5338	do. do.	do.
,, ,,	3962	do. do.	20th October 1898.
,, ,,	4096	do. do.	do.
,, ,,	5349	do. do.	16th December 1898.
,, ,,	5315	do. do.	do.

General Plan of Investigation.

Seven tubes of slant agar were inoculated with the above seven fluids, by allowing ten to fifteen minims of the fluid to flow in each case over the surface of the agar. The necks of the bottles and their mouths and corks were sterilized, and the corks were extracted with sterilized forceps so as to allow no air or organisms to contaminate their contents and nullify the investigations. Two control tubes of the same preparation of agar were kept, into which tubes no Haffkine's fluid was poured, but whose cotton wool plugs were removed for a few seconds, to imitate the process of pouring fluid in, and then re-applied.

All these 9 tubes were kept at the ordinary temperature of the room in a special glass case, and allowed to remain for one day, when they were examined, and it was found that there were growths of living organisms in every one of them except the controls—Nos. 4168 (A), 5324, 3962, and 4096 showed luxuriant growths, the other three less, and No. 5315 showed least growth of all.

Details of the experiments.

No. 1. Haffkine's Fluid: Brew No. 3962.

Ten to fifteen minims poured over agar slant gave after 24 hours yellow white and ivory white discrete colonies all growing on the surface of the agar. There was no cloudiness, gas formation, or liquefaction. Microscopic examination revealed staphylococci in the white colonies, and bacilli and cocci mixed in the yellowish white colonies. The bacilli are short thick rods joined together in places forming chains, some short, others longer, and some are oval like cocci and do not take the stain uniformly. The bacilli are actively motile and the threads lazily motile—decolorized by Gram. The 1st subculture in agar was made of the bacillary colonies (yellowish white), giving as a result yellowish white coalesced colonies, and a few isolated white colonies—these latter consisting of staphylococci and the former of a mixture of bacilli and cocci. A second subculture was therefore made giving also a mixture, but the cocci were fewer in number, so an attenuated third subculture was made with broth and thrown into a petri dish which gave in twenty-four hours a few faint bluish white colonies consisting of bacilli only. This was transferred as a fourth subculture on to

agar slant tubes which gave a pure growth of bacilli only—the growth consisting of a dirty white transparent film. Stained and hanging drop preparations of this film showed the following:—bacilli, fat, straight rods with rounded ends uniformly stained, some single, others joined end to end in pairs, freely motile, the single ones have a wriggling movement, the jointed ones an eel-like movement. The jointed ones appear to be the result of fission in older ones. The bacilli are all decolorized by Gram.

A rabbit (No. 126) was inoculated in the thigh with an emulsion in sterilized broth (4 c.c.) of these bacilli, and a fifth subculture in agar was made and kept. The rabbit (No. 126) had a slight fever (maximum 104·2) for two days and is now well.

No. 2. Haffkine's Fluid: Brew No. 4096.

Fifteen minims of this was thrown over the surface of an agar slant tube and gave after twenty-four hours lemon yellow confluent colonies, white isolated colonies, and white moulds. No cloudiness of agar nor liquefaction nor odour were observed.

Microscopical examination revealed staphylococci in the yellow and white colonies—and mucor mucedo in the mould. No bacilli were present.

No. 3. Haffkine's Fluid: Brew No. 4168 A.

Ten to fifteen minims were poured over the surface of an agar slant tube, and within twenty-four hours there was a feathery greyish white growth—and after forty-eight hours a luxuriant growth of greyish white and yellow expansion. On using a magnifier the colonies are small, circular, and discrete, some being whitish and some yellowish white—at the bottom of the tube a distinct clump of ivory white colonies was seen, small circular and coalescing. No liquefication of agar—all growing on surface.

Microscopic Examination.—By coverglass, stained with gentian violet, the microscope showed staphylococci, micrococci, diplococci, and some bacilli. Hanging drop preparation shows Brownian movement and very feeble motility.

1st Subculture.—A little of the culture was mixed with sterilized broth attenuated and spread over a petri dish of agar, and gave as a result, within twenty-four hours, separate lemon yellow colonies as well as small pearl-like discrete ones and a clump of greyish white colonies. Cover glass preparations showed staphylococci in the lemon yellow colonies, cocci (mono. and diplo.) in the grey white clumps, and bacilli in the pearly blue ones. A second subculture of these pearly blue colonies was made into agar slant tubes with the result that luxuriant growth took place in twenty-four hours, of small pearly blue colonies which coalesced. Cover slip and hanging drop preparations show a short fat coccoid bacillus, straight, very feebly motile, not of uniform thickness, not staining uniformly, with rounded ends, and no general true polar staining; decolorized by Gram's method—some of the bacilli appear like oval cocci.

A rabbit (No. 109) was inoculated with 3 c. c. of a mixture of these bacilli in sterilized broth into the subcutaneous tissue of the thigh, and as a

result had slight fever—maximum temperature 103·6° for two days—no other signs of illness and is now quite well.

A 3rd subculture of the pearly blue colonies of the 1st subculture (petri dish) was made into agar slant and left for three days. When examined the same pearly blue coalesced colonies were seen, which on microscopic examination showed fields of cocco-bacilli which do not stain uniformly and are very feebly motile. A rabbit (No. 110) was inoculated in the thigh with 4 c.c. of this subculture mixed with sterilized broth, and had a rise of temperature to 105·2° for a day, then lysis to normal.

No. 4. Haffkine's Fluid: Brew No. 5315.

Ten to fifteen minims thrown over agar slant gave within forty-eight hours a faint growth of isolated clear colonies—which on microscopic examination revealed staphylococci. No bacilli were found. Rabbits (Nos. 122, 123 and 124) were inoculated subcutaneously with this fluid, re-sterilized by fractional method to 65· C for 1 hour for three consecutive days and gave with No. 122 a maximum temperature of 104·6 the same evening and fever the following two days to 104·2, then fell to normal. Rabbit No. 123 gave 105° same day, next day 106, then lysis to normal. No. 124 gave 105° same evening and 105 next day, then lysis to normal. A rabbit No. 128 was inoculated with the same quantity of the same fluid un-sterilized, and gave 104° the same evening and 104·4 next day.

These Haffkinised rabbits are to be tested as regards their immunity against an inoculation of plague.

No. 5. Haffkine's Fluid: Brew No. 5324.

Ten to fifteen minims of this fluid were thrown over the surface of an agar slant tube. Within twenty-four hours it showed a thick luxuriant growth of greyish white colonies, some the size of a small pin's head; in other parts of the tube a whitish film. All the colonies are growing on the surface of the agar, without liquefaction or odour. Microscopic examination of these colonies by stained cover glass and hanging drop preparations show staphylococci and long bacilli, varying in length, with rounded ends, and straight rods. Some of the rods show swelling in the middle and do not stain uniformly. Hanging drop preparation shows these bulgings very distinctly and the bacilli are feebly motile. The bacilli are decolorised by Gram's method, but the bulging portion retains a little of the stain. They were mainly found in the whitish films of the culture, so the first subculture from this film was made on an agar slant tube which showed a luxuriant growth of whitish expansion as well as a yellow expansion. On microscopical examination these are found to consist of bacilli as well as cocci, so an attenuated second subculture mixed with sterilized broth was thrown on to a petri dish. This showed three distinct forms of growth—a yellow brain-like expansion consisting mainly of staphylococci, a white brain-like expansion consisting mainly of cocci, and isolated grey white colonies consisting mainly of bacilli. A third subculture was therefore made of these isolated grey white colonies on to agar slant tubes. This third subculture shows a pure growth of bacilli which are straight rods, short and fat, somewhat like oval cocci, most of them non-motile, occasionally some of them, joined end to end, show motility.

(*N. B.*—A Subculture of this has been sent to the Plague Commission.)

Rabbit No. 779 was inoculated with 4 c.c. of this third subculture in sterilized broth into the thigh. It gave a rise of temperature to 105° F. for one day and the temperature is 104° F now.

A subculture was made of the yellow brain-like colonies of staphylococci from the second subculture of the petri dishto obtain a pure growth of the organism, and rabbit No. 121 was inoculated in the ear with 1 c. c. of the growth in sterilized broth, giving as a result pyrexia to 104° F. with local œdema, swelling, and congestion, and later an abscess and ulceration.

No. 6 Haffkine's Fluid: Brew No. 5338.

Ten to fifteen minims of the fluid were poured over the surface of an agar slant tube and after forty-eight hours a growth appeared as a thin dewy veil, with a few isolated colonies like dew drops, and a dozen or so of raised round whitish colonies about the size of a small pin's head. No clouding or liquefaction or odour of agar.

Cover glass preparations (hanging drop and stained) were made and showed from the dewy veil and dew drop colonies mainly cocci, while that from the white colonies showed bacilli.

The bacilli were short rods with rounded ends, in length about half the diameter of a blood corpuscle, some are in chains and a few are smaller like oval cocci—they stain fairly uniformly, but are decolorized by Gram's method. They have a very feeble movement in hanging drop—some are motionless. A first subculture of these whitish colonies was made on agar slant giving a fairly luxuriant growth of a yellowish white expansion which shows a mixture of staphylococci and bacilli. A further attenuation by a second subculture (mixed with sterilized broth) was made on a petri dish. The petri dish gave within thirty-six hours bluish white colonies containing long chains of bacilli—which were at once transferred by a third subculture on to an agar slant tube—which gave a growth of yellowish white colonies consisting only of bacilli. These bacilli varied in size, some were small and coccoid, others were in length about half the diameter of a blood corpuscle, and many were seen joined end to end (chiefly in pairs) giving short chains—their ends were rounded and they were rod shaped—showing feeble eel-like movements. A fourth subculture of this was made in agar slant and the remainder of the third subculture was mixed with 4 c.c. of sterilized broth and injected into the flank of a healthy rabbit (No. 120) on the 1st of January. Early on the morning of the 3rd January, within three days of inoculation, the rabbit died—the signs previous to death were firstly a rise of temperature to 103·4, then a sudden fall to sub-normal with giddiness and slight tremors; no diarrhœa. A *post mortem* examination was held the same morning with the following results :—

On reflecting the skin from the abdomen the blood in the vessels as seen to be very dark in color and fluid, with patches of hæmorrhages on the subcutaneous surface of the abdomen and thorax. No enlarged glands were present. *Thoracic organs*—fluid in the pericardium—venous engorgement of the heart—lungs normal in appearance—heart blood dark and fluid. *Abdomen*—petechial hæmorrhages in abdominal wall. Large intestines very dark green in color—spleen not enlarged but very dark. In the regions of the gall bladder, the liver, and adjacent surface of the stomach (which is distended with food) there is bile staining, the kidneys are highly congested and very dark in color. Agar slant tubes were inoculated with a drop of the heart's blood, peritoneal fluid, spleen blood and pulp—one pure agar slant tube being kept as control.

The blood, spleen, liver, and peritoneal fluid of the cadavar were examined microscopically, and bacilli were found in all in abundance, except the peritoneal fluid. The bacilli in all appear to stain uniformly (one or two in a field show polar staining)—varying in length, but generally the length of half the diameter of a blood corpuscle, rod shaped, with rounded ends, some smaller and more oval and some joined end to end, giving a fission like appearance. These bacilli are very similar morphologically to the bacilli in the subculture that was used for inoculation.

A fifth subculture was made from the fourth subculture, and another rabbit was inoculated.

Rabbit (No. 125) was inoculated with 4 c.c. of an emulsion of this subculture in sterilized broth, and had a temperature of 104·4 for one day and then lysis to normal.

The cultures in the agar tubes of the blood, etc., of rabbit No. 120, all show luxuriant bacillian growths except the control which shows none. These bacilli are found to be decolorized by Gram's method. The growth appears to be of the following character:–greyish colonies, the size of pin's heads, circular, coalescing to form a greyish layer–that from the blood shows other kind of colonies raised small circular pearl-like rising above the greyish layer. The growth from the peritoneal fluid seems the purest. On microscopic examination of these growths, the one from the blood shows bacilli, some long rods, rounded ends, which are motile—others smaller, somewhat oval in form which are not so motile, all show irregular staining, and are thicker at the ends than in the centre. The growth from the peritoneal fluid gave small rod shaped bacilli, with rounded ends; and oval bacilli which are very feebly motile and do not stain uniformly. When treated first with alcohol and then stained they show polar staining. The culture from the liver shows two forms of bacilli one set are polar stained, and the other, a longer rod, do not stain at poles. These bacilli are similar morphologically to some of the bacilli found in the subculture that was inoculated into the rabbit, and their culture growths are almost identical. These growths are to be further tested by inoculation into other rabbits, rats, fowls, and pigeons. A subculture of the culture from the rabbit's blood, when put into sterilized broth made the broth cloudy and colonies fell to the bottom as a grey mass. Rabbit No. 125 had a rise of temperature to 104·5 the day following inoculation, and it is now falling towards normal.

A rabbit (No. 129) was inoculated with a subculture made from the peritoneal fluid of rabbit No. 120 that died, and gave a rise of temperature next day to 106·4.

A pigeon was inoculated with a subculture of peritoneal fluid of rabbit No. 120 but only gave a rise of temperature to about one degree.

Two fowls were also inoculated subcutaneously and by injection into the mouth of subculture of peritoneal fluid and spleen of rabbit No. 120 but no pathogenic results followed.

The efficacy of re-sterilizing (by fractional method) and inoculation of this brew of Haffkine's fluid was tried on the following rabbits :—

Rabbit No. 111 was inoculated subcutaneously with 20 c.c. of the unsterilized fluid and showed a temperature of 106° (maximum) the second day

Rabbit No. 779 was inoculated with 4 c.c. of this third subculture in sterilized broth into the thigh. It gave a rise of temperature to 105° F. for one day and the temperature is 104° F now.

A subculture was made of the yellow brain-like colonies of staphylococci from the second subculture of the petri dishto obtain a pure growth of the organism, and rabbit No. 121 was inoculated in the ear with 1 c. c. of the growth in sterilized broth, giving as a result pyrexia to 104° F. with local œdema, swelling, and congestion, and later an abscess and ulceration.

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A fifth subculture was made from the fourth subculture, and another rabbit was inoculated.

Rabbit (No. 125) was inoculated with 4 c.c. of an emulsion of this subculture in sterilized broth, and had a temperature of 104·4 for one day and then lysis to normal.

The cultures in the agar tubes of the blood, etc., of rabbit No. 120, all show luxuriant bacillian growths except the control which shows none. These bacilli are found to be decolorized by Gram's method. The growth appears to be of the following character:–greyish colonies, the size of pin's heads, circular, coalescing to form a greyish layer–that from the blood shows other kind of colonies raised small circular pearl-like rising above the greyish layer. The growth from the peritoneal fluid seems the purest. On microscopic examination of these growths, the one from the blood shows bacilli, some long rods, rounded ends, which are motile—others smaller, somewhat oval in form which are not so motile, all show irregular staining, and are thicker at the ends than in the centre. The growth from the peritoneal fluid gave small rod shaped bacilli, with rounded ends; and oval bacilli which are very feebly motile and do not stain uniformly. When treated first with alcohol and then stained they show polar staining. The culture from the liver shows two forms of bacilli one set are polar stained, and the other, a longer rod, do not stain at poles. These bacilli are similar morphologically to some of the bacilli found in the subculture that was inoculated into the rabbit, and their culture growths are almost identical. These growths are to be further tested by inoculation into other rabbits, rats, fowls, and pigeons. A subculture of the culture from the rabbit's blood, when put into sterilized broth made the broth cloudy and colonies fell to the bottom as a grey mass. Rabbit No. 125 had a rise of temperature to 104·5 the day following inoculation, and it is now falling towards normal.

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A pigeon was inoculated with a subculture of peritoneal fluid of rabbit No. 120 but only gave a rise of temperature to about one degree.

Two fowls were also inoculated subcutaneously and by injection into the mouth of subculture of peritoneal fluid and spleen of rabbit No. 120 but no pathogenic results followed.

The efficacy of re-sterilizing (by fractional method) and inoculation of this brew of Haffkine's fluid was tried on the following rabbits :—

Rabbit No. 111 was inoculated subcutaneously with 20 c.c. of the unsterilized fluid and showed a temperature of 106° (maximum) the second day

then lysis to normal, fever lasting four days. Rabbit No. 125 inoculated with the same quantity of re-sterilized H. F. 5338 subcutaneously, showed temperature of 104·8 (maximum) for a day.

No. 7. Haffkine's Fluid No. 5349.

Ten minims of this fluid thrown over agar slant gave within forty-eight hours isolated greyish white colonies and a whitish scum over the agar—no liquefaction or odour—later a few isolated white colonies appeared.

Microscope revealed only micrococci and staphylococci in the isolated white colonies. No bacilli were found.

Conclusions arrived at as the result of the experiments up to date.

The samples of Haffkine's fluid examined bacteriologically all show contamination with living micro-organisms—in some cases, Nos. 5315, 5349 and 4096, the contamination seems to be with cocci alone, in the others with a mixture of cocci and bacilli. Although the samples taken show the above contamination, other bottles of the same brew show contamination with other forms of micro-organisms. As far as the investigations have gone it will be seen that the micro-organisms isolated from the fluids are many of them distinctly pathogenic, some more virulently so than others, in one case (No. 5338) the microbe was so pathogenic as to cause death (*vide* rabbit 120). Rabbit No. 121 shows the effect of the contamination with cocci, which was the staphyloccus aureus, producing local disease (abscess and ulceration) and a pyæmia. If the temperature charts of the inoculations with one of the brews and the organisms isolated from that brew be compared, it will be seen that the inoculation with the fluid as received from Bombay, gives a greater rise of temperature in most cases than the pyrexia produced by the isolated organisms—therefore, if the living organisms were extracted from the fluid before inoculation, a lower temperature ought to be the result of inoculating such a fluid. This idea seems to be confirmed when the temperature charts of rabbits 101 and 105 are compared. No. 101 is the result of inoculation of Haffkine's fluid No. 4168 giving a rise of temperature to 106°, followed by death; whereas No. 105 shows the result of inoculation of the same of Haffkine's fluid re-sterilized giving a resulting temperature of 104·5 as the maximum, and instead of death recovery. This is one example of the effect of resterilizing Haffkine's fluid, but others are now being carried out. It would appear, therefore, that re-sterilizing does away with a severe form of septic fever and complications which arise from the contaminating organisms; but the question whether this re-sterilizing destroys the efficacy of the original Haffkine's fluid remains to be considered, this is also being done—*vide* temperature chart of rabbit No. 105 (Haffkinized with brew No. 4168). One C.C. of plague culture was inoculated on the 1st January, giving a slight ~~reactionary~~ fever only, whereas the control rabbit No. 115 inoculated on the same day with the same dose of the same culture, gave a rise of temperature to 107 and death. The re-sterilized Haffkine's fluid was tested as regards the presence of any living organisms at the same time as inoculation was done, but no living organisms were found

to grow on the agar tube. Temperature charts of rabbits No. 122, 123, 124 and 128 $\frac{6}{30}$ can be compared also—with regard to the question of re-sterilizing, but it ought to be borne in mind that this brew of Haffkine's fluid showed only slight contamination of cocci.

The following deductions may be drawn from the experiments that have been done so far:—

1stly, that in bottling the Haffkine's fluid from the retorts sufficient care does not seem to have been exercised in preventing contamination.

2ndly, allowing that some contamination is unavoidable, due to the enormous out-turn of fluid necessary to meet the demand, all the bottles on being filled and before being sent away should be re-sterilized to 60·C for an hour, and the corks should be replaced by proper fitting glass stoppers. This, ~~however,~~ probably would not destroy the chemical products of putrefaction. If this were done, and if it was proved that re-sterilizing does not injure the protective influence of the inoculation fluid, then I feel sure there would be less number of cases of septic poisoning among the inoculated subjects, for the fluid which is received now from Bombay is undoubtedly a "putrescent organic liquid" as stated before the Commission by the Plague Commissioner, Hyderabad.

Further investigations and experiments with reference to the inoculation fluids and their isolated living organisms are being carried out by the authorities in charge of the laboratory in Hyderabad in continuation of the above work, and if so required, a further report at a later date can be furnished by them.

My thanks are due to Lt.-Col. E. Lawrie, I. M. S., for allowing me to use the laboratory; and to his staff for their kind assistance during the above work.

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PLAGUE—HYDERABAD STATE.

Investigations Tabulated.

Bactirioscopic Examination of Haffkine's Fluid.

No. 1.

Agar slant.
+
H. F. No. 4168 A.

Luxuriant growth, a mixture of cocci and bacilli

1st subculture.
Attenuated in broth; and a petri dish inoculated.

3 separate colonies.

A. Lemon yellow = staphylococci.
B. Grey white = cocci { mono—
                      { diplo—
C. Pearly blue = bacilli.

C

2nd Subculture agar slant. One day's growth.

Bacilli, pure growth of pearly blue circular colonies coalesced.
Bacilli, short fat, (cocco-bacilli) feebly motile.

Rabbit No. 109 was inoculated with 3 c.c. of this growth mixed with broth. Result fever slight (103·6 max.) for 3 days—now alive and well.

3rd Subculture agar slant. Three day's growth.

Bacilli, pure growth, pearly white coalesced colonies, mainly cocco-bacilli.

Rabbit No. 110 inoculated. Result, fever 4 days max. temp. 105·2, now alive and well.

No. 2.

Agar slant.
+
H. F. No. 5324.

Luxuriant growth of Staphylococci and bacilli.
1st subculture.

Attenuated by subculture in agar slant.

2nd subculture with broth.
(control of broth, done 3-1-99.)
Attenuation on to petri dish.

Three kinds of growth.
A. Yellow brain-like growth = cocci.
B. White do. do. = cocci.
C. Isolated grey-white colonies = bacilli.

C. Bacilli.

3rd Subculture into agar slant, one day's growth.

Luxuriant pure growth of whitish grey film of bacilli.
Bacilli = short fat coccus-shaped, spore formation seen, motile at times but chiefly non-motile.
(A special subculture of this was sent to the Plague Commission.)

Rabbit No. 119 inoculated with 4 c.c. of this pure subculture: result fever, max. temp. 105°.

A. and B. Cocci.

Subculture in agar of pure staphylococci.

Rabbits ear (121) inoculated with above, gave local abscess and ulceration.

No. 3.

Agar slant.
+
H. F. 5338.

Growth, mixture of cocci and bacilli.

Subculture in agar slant.

Luxuriant growth of cocci and bacilli.

Attenuated 2nd subculture in petri dish.

Yellow and white colonies of staphylococci.
Blue white colonies of bacilli.
Bacilli = irregular size, some thin, others thick, jointed and oval, (motile).

*A subculture (4th) kept and a rabbit No. 120 inoculated with 4 c.c. of culture in broth.

Death within 60 hours, temp. sub-normal.

Cultures in agar were made of spleen liver and peritoneal fluid of the rabbit, giving growth of bacilli similar to the bacilli in the sub culture.

* The subculture shows thickish bacilli motile growing by fission.

No. 4.

Agar slant
+
H. F. No. 3962.

Luxuriant growth, mixture of cocci and bacilli; cocci = mono, diplo, & staphylococci.

Subculture in agar slant.

A. Yellow coalesced colonies = bacilli & cocci.

B. White isolated colonies = staphylococci.

2nd Subculture in agar.

Bacilli chiefly, also a few cocci.

3rd. Attenuated subculture with broth on to petri dish—shows gossamer veil expansion over which a thick yellow white expansion is present.

4th. Subculture on agar slant gives luxuriant growth of yellow white colonies of bacilli similar morphologically to bacilli from 5338.

Subculture on agar slant of liquid yellowish expansion.

Subculture on agar slant.

Rabbit No. 126 inoculated with 4 c.c. of bacilli subcutaneously, result slight fever, now well.

No. 5.

Agar slant.
+
H. F. No. 4096.

Luxuriant growth of—
Lemon yellow colonies = Staphylococci.
White = Staphylococci.
Moulds whitish = Mucor mucedo.
No bacilli found.

No. 6.

Agar slant.
+
H. F. No. 5349.

Growth of isolated grey white colonies of micro-cocci only.
One or two white isolated colonies of staphylococci.

No. 7.

Agar slant.
+
H. F. No. 5315.

Faint growth of clear hyaline like colonies of staphylococci.
No bacilli.

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for perusal and return.

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Recended.