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The bactericidal action of the waters of the Jamuna and Ganga rivers on Cholera microbes

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Crossing the Ganga or the Jamuna that flows through big Indian cities, when one sees thousands of inhabitants washing themselves, their cattle and their clothes in the dirty cloudy waters and one thinks of the half-burnt corpses that often have their final journey in the river, one could be excused for thinking that these waters should be quite dangerous for consumption and that the worship of these sacred rivers by the Hindus proves their ignorance of all idea of health and cleanliness. The European authorities seem to think so, and as regards the spreading of Cholera, they would like to consider that the Ganga is the main cause for transmitting the disease in its country of origin, and the foster father of the microbe.

However, a simple microscopic examination of the waters of these two rivers reveals a noticeable difference compared to the equally cloudy waters of European rivers. The latter are found to have an abundance of plant and animal matter, a lot of germs and living animal and plant forms. Water from the Ganga and Jamuna, on the other hand, does not contain any trace of organic matter, unless it has been collected from near a bathing ghat downstream from the city. The silt brought by the river is almost exclusively formed of sand or mica. Bacteriological examination shows that microbes are much more scarce than those found in the major rivers of Europe (1). Our rivers are

(1) *About microbes in Indian rivers*. Communication at the Indian Medical Congress held in December 1894.

Our rivers are often free from aquatic plants and any other form of plant life.

A closer examination reveals several reasons for this comparative bacteriological purity. India does not have a big sewage network that pours out into the river. Of course big cities have sewers above the ground, but for a large part of the year, the sewage water is negligible. It is rare to find, as in Europe, chemical or other factories situated on the banks of rivers that contribute to the pollution of river water.

The best protection of river waters in India in the region that I am most familiar with (the central part of the Gangetic plain), is due to the fact that their banks are fringed with stretches of barren area, often one to two miles wide, cut across by ravines, with very few villages. The river, once it has been polluted by a big city, remains free from any new pollution until it reaches the next big city, which is sometimes situated 200 miles away. I have seen that within the limits of the North Western Provinces, the Jamuna becomes purer, the farther it flows from its source. There are only two villages situated on its banks for 12.5 miles downstream from Agra. There are only three villages for 23 miles upstream, and without having specific numbers, I believe that none of these villages have more that 500 inhabitants.

The self-purificatory power, which depends on the action of air or light, should no doubt be much more active in India than in Europe. The wide rivers of the N-W provinces flow in thin meandering layers amidst sand banks and are exposed to the action of light and oxygen under the most conducive conditions, this is helped by temperatures being higher than that in Europe.

In addition, for a large part of the year, the source of this river water is not from rain or surface drains but from the melting of the snows of the Himalayan peaks, which is free from any microbes. The source of these rivers is even purer and free from germs compared to European rivers, fed by rainwater, which has been washed down from the soil surface.

At the start of the last days of the hot season, with the river being unusually low, I found in the Jamuna:

56 to 76 microbes per cc at 5 miles upstream from Agra.
700 to 750 microbes per cc opposite the water supply point.
3000 to 25000 microbes per cc opposite the city and downstream.

From this point, the number of microbes started decreasing rapidly up to a distance of 12.5 miles where the figure was not more than 128 to 130 germs per cc. Later, during the hot season (11th April), the river being very low with very mild currents, the number of microbes in the water sample collected downstream from the city was always more than 100,000 per cc and the exact number could not be estimated. But three miles further downstream, this was not more than 90 or 100, and at 12.5 miles from the city, this was only 26 to 80.

Here are the figures found per cc, at the water supply point from hydraulic pumps at various times of the year: each figure is the average of at least three readings.

January	1,680]	cold season and mild rain
February	1,084]	from time to time
March	1,133]	
April	580]	
May	662]	hot and dry weather
June	725]	
July	2,900]	
August	3,140]	rainy season
September	1,033]	
October	2,183]	
November	850]	cold and dry weather
December	1,016]	

Some impurities from a village and other sources reach the river at the level of the water supply point. The purity should hence be lower than the observations mentioned above and made at 5 miles upstream.

II

These observations on the bacteriological purity of the Ganga and its tributaries do not shed any light on what has been for long the main objection, from experiences in India, to the idea that cholera could be a water-borne disease. The basic law concerning the progress of widespread epidemics in India is that, having originated in Bengal, they go upstream the river and its tributaries.

One has never seen epidemics coming downstream the Jamuna and the Ganga. How is this possible, if cholera is water-borne? How is it possible that when cholera breaks out at a place of pilgrimage on the banks of one of these rivers, it does not affect the villages situated downstream, unless the infection is borne by returning pilgrims?

It is not possible to explain this extraordinary fact by assuming that the cholera germ is not borne by the river water. Of course, a native would never use the banks of the river for his ablutions unless absolutely necessary, as he regards these waters as sacred (1). But in the absence of this source of pollution, there are many others. Let us first talk about the drainage water in big cities. I have found cholera germs present in the water of one of the drains in Agra that flows into the river. Another source of contamination is due to washing of clothes and people bathing in the river. Several times (2), I have found the cholera microbe present at bathing spots in the Jamuna and Ganga, during pilgrimages at times of festivals in Allahabad and other cities. But the most apparent mode of infection is the practice of throwing corpses of cholera victims into the river. Normally, these corpses are partially burnt before being thrown into the sacred river. But in many districts, corpses of cholera victims are not consigned to the purificatory effects of the fire beforehand. As far as I could make out, this custom is due to the fact that death by cholera seems like a judgment of God: the victim is considered to be “condemned beyond redemption” and it is a matter of losing one’s faith rather than burning the cadaver.

It seemed to me that the above facts could perhaps be because the microbes were unable to survive in the waters of the Ganga or the Jamuna, which could not sustain the life of the cholera microbe due to lack of nutritional matter. The following experiments were carried out to verify this possibility and led me to discover that these waters contained an antiseptic that had a powerful bactericidal action on the cholera germ.

(1) The natives have no scruples about squatting down at a little distance from the banks. Their excrements are rapidly removed by animals or dried and rendered harmless under the Indian sun. But the riverside is free from any pollution of this kind. However, cucumber plants are sown on the sand banks left to dry in summer and fertilised with human excrements. This is one of the sources of contamination of river water.

(2) Observations on cholera in India. Indian Medical Gazette, May, 1895.

This bactericidal action was actually very slight during the first tests, because, as I realised later, I used to sterilise the water by heating it in an autoclave. This is what the following example indicates. In this and the following experiments, the word "boiled" refers to boiling at 115 Degrees in an autoclave for a half hour. To water obtained from different sources which has been heated in this manner, cholera germs were added and cultured immediately after addition and at different intervals. The figures obtained were:

Water from	Number of colonies after ..... hours							
	0	1	2	3	4	5	24	28
Jamuna boiled	12,500	20,000	17,500	30,000	32,000	26,000	4,000	5,000
From the tap	14,000	17,000	17,500	21,000	20,000	19,000	17,000	15,000
Ganga	10,000	8,000	12,000	5,000	13,000	20,000	18,000	15,000
Zem-Zem	10,000	21,000	28,000	50,000	60,000	90,000	80,000	100,000

It is seen that in the water from Jamuna and tap water from Agra (which is Jamuna water), there is no marked destruction of microbes whereas there is a slight increase in the two last water samples. Water from the famous spring at Zem-Zem, the sacred spring in Mecca, showed that the number of cholera microbes became ten times more than in 24 hours. Here are the differences obtained by using filtered water instead of heating it in an autoclave.

Water from	Number of colonies after ..... hours						
	0	1	2	3	4	25	49
Ganga filtered	5,500	3,500	200	0	0	0	0
Ganga boiled	6,000	5,000	6,000	6,400	4,000	3,800	15,000
Ganga boiled and filtered	7,000	8,000	8,000	7,500	6,000	3,000	-
Wellwater filtered	8,500	8,000	7,500	10,000	80,000	4,000	15,000
Wellwater boiled	7,500	10,000	12,000	14,000	16,000	30,000	25,000

It is seen that the unboiled water of the Ganga kills the cholera germ in less than 3 hours. The same water, when boiled, does not have the same effect. On the other hand, well water is a good medium for this microbe, whether boiled or filtered; the Pasteur filter was used in these experiments as well as the following ones. In each experiment, several control observations were made to prove the purity of the cultures and sub-cultures.

The following shows that the bactericidal effect is not due to absence of nutritional matter in the water.

Water from	Number of colonies after ..... hours					
	0	1	2	3	4	24
Jamuna filtered	4,200	800	0	0	0	0
Distilled water	4,500	5,000	6,000	5,500	200	12,000

However, I have often noticed that cholera microbes die in distilled water but never as quickly as in the waters of the Ganga or the Jamuna. I would usually sterilise the water I used by filtration. During one experiment, I added cholera germs to unsterilised Jamuna water to check if the bactericidal properties were not caused by this treatment, and found that by using the ordinary method of culturing with peptone, they died in less than four hours.

To give the benefit of doubt to the cholera germ, in the following experiments, I used a culture in Jamuna water that was sterilised and traces of peptone and a little alkali added to it. This medium was sown 2 to 3 days before the experiment, by making a new culture every day. This was done to try to acclimatise the microbe in the water of the Jamuna river, to avoid a rather abrupt change from a rich culture medium into water lacking in nutrients like this river water. But the cholera microbe is not very sensitive to changes. The typhoid bacillus is more sensitive, as shown by Mr. Haffkine (Annals of the Pasteur Institute, t.iv. page 363, 1890). Nevertheless, this bacillus is not killed by immersion in Jamuna water, under laboratory conditions, as proved by the following experiments:

	No. of colonies after ------ hours					
Typhoid bacillus acclimatised in the broth and then placed in	0	1	2	3	4	24
Well water 20,000	500	100	100	150	100	
Tap water 15,000	250	50	100	50	50	
Typhoid bacillus acclimatised 50,000	800	600	100	200	250	
in the broth and then placed in 90,000	900	100	200	150	100	

The typhoid bacillus had been acclimatised in the broth or in the water, three days before the experiment, with fresh cultures being made every day. The tap water was from the Jamuna, as mentioned earlier, and usually has a bactericidal effect on the cholera germ.

I have already mentioned the effects of heating on the water from the Ganga river. This is the effect of water from the Jamuna that was added to cultures of the second Haffkine vaccine.

	No. of colonies after ------ hours						
Water from	0hr	1hr	2hr	3hr	4hr	25hr	49hr
Jamuna filtered	2500	1500	1000	0	0	0	0
Jamuna filtered	5000	4000	3000	3000	2000	0	0
Jamuna boiled and filtered	5000	4000	6000	5000	6000	10000	36000
Jamuna boiled	6000	5000	4500	4000	4000	3000	8000

The above experiments have been done with cultures of the second Haffkine vaccine. It was necessary to know if a similar bactericidal action of the waters from the Ganga and Jamuna was manifested on the Indian cholera microbes. For this purpose, I used a microbe from an epidemic that broke out in Bellary (Madras Presidency).

Bellary cholera	No. of colonies after hours					
Water from	0hr	1hr	2hr	3hr	4hr	25hr
Jamuna filtered	8000	4000	3000	100	0	0
Jamuna filtered	6000	5000	1500	0	0	0
Jamuna filtered	9000	5000	1000	160	0	0
Tap filtered	7000	1200	100	0	0	0
Tap filtered	8000	1000	200	0	0	0
Ganga filtered	8000	8000	6000	8000	12000	21000
Ganga filtered	6500	7000	7000	1000	12000	24000
Haffkine vaccine						
Jamuna filtered	10000	3000	150	0	0	0
Jamuna filtered	8000	2000	100	50	0	0
Jamuna filtered	10000	1500	150	100	0	0
Tap filtered	7500	3000	150	50	0	0
Tap filtered	7000	4000	100	0	0	0
Ganga filtered	9000	3000	1250	4000	2000	20000
Ganga filtered	8000	5000	4000	2800	3000	25000
Control. Well water						
Cholera of Bellary	7000	8000	10000	10000	20000	18000
Cholera of Bellary	8000	10000	11000	10000	16000	16000
Haffkine vaccine	9000	8000	3000	4000	9000	28000
Haffkine vaccine	8000	8000	2500	2000	6000	25000

In this experiment, the Jamuna water killed the cholera microbes introduced into it, whether it was taken directly from the river or first subjected to filtration through sand and then passed through an Anderson purifier. The effect was the same on microbes from cholera from Tonkin or from the Bellary epidemic. On the other hand, Ganga water did not have any effect in any of the cases. To avoid all the causes of error, all the tubes containing the water specimens were those that had recently arrived from Europe, new and just washed in tap water. I have often taken this precaution although there wasn't any reason to think that this would be absolutely necessary.

In another case, even the Ganga water did not show any effect. I think that this is because of the long train journey and that 50 to 60 hours have elapsed from the time the water is collected to the time studies are conducted. The following experiment shows that Jamuna water slowly loses its effect on the cholera germ. The samples said to have been preserved were taken several hours before the experiment.

	No. of colonies after _____ hours						
Water preserved in a bottle	0h	1h	2h	3h	4h	29h	53h
Tap filtered	3000	1800	15000	1500	1800	7000	
18000							
Tap boiled	3600	3000	800	4000	750	10000	
22000							
Water preserved in tinplate							
Tap filtered	3000	1300	1100	1000	700	24000	
8000							
Tap boiled	2900	3200	2800	3000	3100	36000	
30000							
Fresh sample of water							
Tap filtered	4000	1500	750	250	50	0	0
Tap boiled	4000	4000	5000	5100	5000	6000	
18000							

One could object to these experiments by saying that, perhaps, the microbes have not been killed by the Ganga or Jamuna waters, only a bit modified, so they are not capable of reproducing and forming colonies in agar-agar. To avoid such an objection, many a time, I have added some peptone and alkali to the apparently sterile mixture of Jamuna water and cholera microbe, about 5 to 24 hours after preparation of the mixture. I had seen earlier that this mixture removes all bactericidal properties exhibited by the Jamuna waters on the cholera microbe, and the other point is that the peptone solution is the best culture medium that we know of for this microbe. The tubes containing Jamuna water that was treated in this manner remained sterile, thus proving that the cholera microbes had been killed. Two to three days later, these tubes were seeded with cholera microbes and produced a good culture. The medium was hence favourable.

I have shown that Jamuna water loses its power on heating. Hence, one wonders if this bactericidal substance is destroyed by heat or if this is a volatile substance that is eliminated from the liquid. To know this, I heated the Jamuna water in hermeticallly sealed tubes; the water should lose all bactericidal properties if there is any destruction by heat, and retain these properties if this is due to any volatile substance that cannot escape, as the tube is opened only after cooling. It is the latter that was found to occur, as shown by the following experiment.

	No. of colonies after _____ hours							
Water from	0h	1h	2h	3h	4h	5h	24h	48h
Jamuna heated in sealed tube	2100	150	50	0	0	0	0	0
Jamuna heated in sealed tube	1500	50	0	0	0	0	0	0
Jamuna filtered	1000	450	300	350	50	0	0	0
Jamuna filtered with 1 drop of NaCO3 at 10/0	1500	900	200	100	250	300	1200	0
Jamuna heated in open Tube	1800	1000	1250	600	1900	1500	3800	2500
Well water, filtered	1000	800	500	750	800	900	1800	1000

Similar results in the following experiment:

	No. of colonies after _____ hours					
Water from	0h	1h	2h	3h	4h	24h
Jamuna heated in sealed tube	4200	1109	0	0	0	0
Jamuna heated in sealed tube	3500	100	0	0	0	0
Jamuna heated in open tube	4000	3500	5000	4500	5000	22000
Jamuna heated in Platinum capsule	5000	3750	4000	5000	4500	2000
Distilled water	4500	4000	6000	5500	200	12000
Jamuna filtered	4200	800	0	0	0	0

These two experiments show that Jamuna water heated in closed vessels is capable of killing cholera microbes, and it loses this property when heated in a long-necked vessel closed with cotton or in a rather deep platinum capsule, as used in this experiment.

It also loses this property on slight alkalinisation with Sodium Carbonate.

In the second of these experiments, the water was obtained from Kailasi-Ghat, a locality situated about 22 miles upstream from Agra. Hence, it is not only the water from the vicinity of the city that possesses this property; besides, my experiments were carried out at all times of the year except during the monsoons.

During some seasons, all the water from the Jamuna flows into the Agra-Delhi canal, 200 miles upstream from Agra. This is done so well that the joints of the structure at the lock in Delhi are caulked; so as not to allow a single drop of the precious liquid to escape. All the water found in the river in Agra is thus surface water. The bactericidal property is still present in this water, as when there is a mix of the water from the melting snows of the Himalayas. This surface water is the same water that feeds the wells, which does not have this bactericidal property, as seen earlier. Hence, the antiseptic property normally present in this water is neither from the water from the melting snows nor from the surface water, this is due to an unknown substance formed in the river or formed in situ by the water. The same substance seems to be present in the Ganga. It is highly improbable that this substance exists in the many streams of the Himalayas, of the Central Provinces or of the Madras Presidency, where it is known that the water could transmit infections.

I could not add anything more about the nature and origin of this substance; I only know one thing, that this substance is volatile.

I thought it was important to know if this bactericidal activity of the Jamuna water was the same upstream and downstream from the city, i.e., if this was influenced by all that was emptied into the river by the city. During the winter of 1895-1896, I found a very conducive occasion to carry out this study; the river was abnormally low due to insufficient rains, so much so that the water supply had been affected. The current was hence very slow and the pollution from the city, washing ghats, baths, drains and the melon beds was much more concentrated than usual. A system of paving with flow of surface water introduced in some areas of the city increased the input into the drains. Water from one of them contained 8 millions of microbes per c.c.

It also seemed useful to check if the corpses that were constantly thrown into this river at the cremation site downstream from the city had an effect on the bactericidal property of the water.

At the time of carrying out the experiment, a type of influenza caused an increase in the mortality rate of the city, and the current was too slow to carry the corpses at the normal speed. It was not unusual to see 8 or 9 of them floating over a distance of a few hundred metres.

These conditions were evidently favourable to the experiments. I collected water samples upstream from the city, then upstream and downstream from the place where bodies were cremated. It was quite easy to collect water samples from the area near a corpse submerged in the water for some time. But nothing could be more difficult than collecting samples from near a corpse that had just been thrown into the water. I waited for the drop in my boat, and pushed towards the corpse as fast as possible. I am sure I reached a minute or a minute and a half after a half-burnt body was thrown in. But four large turtles were already attacking it, so busy with their meal that they took no notice of the powerful blow that I dealt them with a heavy bamboo and I could push them away only after ten minutes. I pushed the body onto a shoal and when it had run aground, I plunged my flask near it such that the water that was collected contained all the effluvium coming out of the body. Although the turtles had been driven away as quickly as possible, the head, arms, the viscera and the lower part of the legs were already missing. This shows what wonderful undertakers these turtles are! If the corpse's head had not been broken during the funeral, it remained intact. But it is usually missing in the floating bodies. Soon after they are thrown into the water, not only are the parts mentioned above, removed by the turtles but also whatever muscular mass of the trunk that can be bitten off by them. There is no rotting smell except in the skull. If the body runs aground onto a sandy shoal, vultures and other carnivorous birds clean it up to the bones.

I put a pipette into the muscular mass of an old corpse, near which I had collected some water and carried out a bacteriological study of this sample. It contained 40,000 microbes per c.c. If the corpse had been in the Thames, the number would have probably been considerably more than this.

The water flowing near the corpse at this moment contained about 20, 000 microbes per c.c., due to the impurities from the city, such that the contamination from the corpses in the vicinity of Agra seems to me to be negligible, and it is only from a sentimental point of view that the practice of throwing half-burnt bodies in the river should not be recommended.

Whatever accompanies this custom is repulsive to our European tastes. Fortunately, during this unpleasant adventure, due to my desire to collect water before even the relatives of the dead had the time to recover from their astonishment, I hardly paid any attention either to the vultures circling above my head, or to the turtles, about one metre in length, swimming on the bank with bits of human flesh in their mouth, or the smell of burning bodies. The following results show that no practical objection can be made to this custom.

	No. of colonies after _____ hours						
Water from	0h	1h	2h	3h	6.5h	21h	45h
Jamuna upstream the city	1200	200	0	0	0	0	0
Jamuna downstream the city	1500	0	0	0	0	0	0
Jamuna near an old body	1250	50	6	0	0	0	0
Jamuna near a new body	2000	500	200	0	0	0	0
Jamuna upstream the city, boiled 25000	1250	1200	1500	200	1000	2000	
Jamuna downstream the city, boiled 6000	1000	2000	500	800	1000	13000	
Well, boiled 16000	1200	1250	1700	1200	1500	3000	

Hence it seems that all impurities from a big city, as well as the practice of throwing half-burnt bodies in a river have no effect on the property of the Jamuna river of destroying cholera microbes.

III. CONCLUSION

Although the scientific interest of the preceding results maybe limited by the fact that I have not yet discovered the nature and origin of the antiseptic substance present in the waters of the Ganga and Jamuna rivers, what appears interesting is that they explain why cholera does not travel downstream rivers in India. Besides, they have practical applications, as I have shown in a book (1) that I have just published on cholera. To cite an example that may be of interest to European readers, the results suggest that during Hindu pilgrimages to sacred places on the banks of the Ganga and the Jamuna, it would be better to advise against the use of well water and encourage use of river water. This would no doubt be easy, as the pilgrims regard this water to be sacred as well as a stimulant for digestion. I believe that much of the dangers of annual pilgrimages to famous and big fairs as in Hardwar, could disappear by closing 5 or 6 wells that exist at present.

(1) Cholera in Indian Cantonments, published by Deighton-Bell and Co., Cambridge, England.