

COMPARATIVE BACTERIOLOGICAL STUDIES

By Prof. F. Marzullo - Division of Clinical Research - Istituti Ospedalieri - Modena

Studies have been carried out on behalf of CISP the Italian Vitreous Enamel Association on the bacteriological properties of surfaces of various materials, both native and specially treated, and stored in a refrigerator. The studies were intended to show

- 1) The level of spontaneous infection in contact with milk, broth or meat;
- 2) The level of infection provoked by contact with known bacterial cultures;
- 3) The ease of decontamination by physico-chemical agents

The following materials were examined:

1. Stainless Steel
2. Anodized aluminium
3. Native aluminium
4. Painted aluminium
5. Enamelled sheet iron
6. Painted sheet iron
7. ABS Polymer
8. Polystyrol
9. PTFE

Materials and Methods

Nine types of plates were used (50x50x12.5 mm).

They were sterilized by UV-irradiation and placed in Petri dishes which had been sterilized by heating.

A) Spontaneous infection

This was followed by studying the development of the spontaneous bacterial flora present on meat, milk and broth. The bacterial load of the three materials was determined initially and after 24-48-72 and 144 hr.

B) Provoked infection

was followed by studying the development of colonies of known titre (i.e. number of organisms) of *Staphylococcus pyogenes*, *Escherichia coli* and *Proteus mirabilis* for 24, 48, 72 and 144 hr.

C) Ease of decontamination

This was studied by following bacterial growth (at 24, 48, 72 and 144 hr.) on surfaces treated with soap and water; water and acetic acid; water and bicarbonate, applied with a brush or cloth.

A cloth or soft brush was preferred so as to avoid scratches which would prevent thorough cleaning and so favour the retention of bacteria.

This phenomenon was separately studied and we showed that even minimal abrasions led to this, particularly with surfaces of ABS, painted iron, polystyrol, painted aluminium and PTFE.

There was little effect with stainless steel or anodized aluminium and none on enamelled iron. In order to give a better picture of any changes, the foodstuffs tested (milk, broth and meat) were first held in an incubator at 37°C until a sufficiently high bacterial level was reached (900 x 10⁷ c.c.). Fifteen tests were carried out for each investigation.

1st EXPERIMENT - SPONTANEOUS INFECTION

Virtually identical results were obtained with milk, broth and meat and they are therefore unified. Table 1 gives the mean bacterial levels found in the 15 tests.

TABLE 1

SURFACE	Bacterial level (x 10 ³)				
	0 ore	24 ore	48 ore	72 ore	144 ore
Stainless steel	5.030	3.050	3.090	3.030	3.020
Anodized aluminium	5.070	3.900	4.010	3.900	4.000
Native aluminium	5.070	3.090	3.090	4.050	3.000
Painted aluminium	5.030	4.000	3.070	3.050	2.900
Enamelled steel	5.070	2.015	2.000	2.020	2.020
Painted iron	5.070	3.100	3.060	3.060	3.060
ABS polymer	5.070	3.025	3.070	3.090	3.025
Polystyrol	5.070	4.000	4.000	4.070	3.090
PTFE	5.080	3.000	3.000	3.000	3.000

N.B.: The figures are in thousands

2nd EXPERIMENT - PROVOKED INFECTION

Here too the results were virtually constant and those from the 15 tests are averaged in Tables 2, 3 and 4.

TABLE 2

SURFACE	STAPHYLOCOCCUS PYOGENES - 10^{-3} x no. cells at				
	0 ore	24 ore	48 ore	72 ore	144 ore
Stainless steel	200.000	50.000	40.000	30.000	20.000
Anodized aluminium	200.000	50.000	50.000	40.000	20.000
Native aluminium	200.000	45.000	45.000	40.000	35.000
Painted aluminium	200.000	30.000	15.000	15.000	7.000
Enamelled steel	200.000	15.000	5.000	1.000	650
Painted iron	200.000	20.000	20.000	10.000	10.000
ABS polymer	200.000	30.000	28.000	20.000	10.000
Polystyrol	200.000	20.000	15.000	15.000	7.500
PTFE	200.000	60.000	60.000	60.000	50.000

N.B.: The figures are in thousands

TABLE 3

SURFACE	ESCHERICHIA COLI - 10^{-3} x no. cells at				
	0 ore	24 ore	48 ore	72 ore	144 ore
Stainless steel	300.000	75.000	50.000	30.000	25.000
Anodized aluminium	300.000	68.000	60.000	18.000	18.000
Native aluminium	300.000	70.000	60.000	50.000	50.000
Painted aluminium	300.000	40.000	20.000	16.000	9.000
Enamelled steel	300.000	18.000	10.000	5.000	5.000
Painted iron	300.000	40.000	40.000	10.000	10.000
ABS polymer	300.000	30.000	30.000	30.000	18.000
Polystyrol	300.000	26.000	14.000	14.000	10.000
PTFE	300.000	75.000	50.000	50.000	50.000

N.B.: The figures are in thousands

TABLE 4

SURFACE	PROTEUS MIRABILIS - 10^{-3} x no. cells at				
	0 ore	24 ore	48 ore	72 ore	144 ore
Stainless steel	400.000	100.000	90.000	60.000	50.000
Anodized aluminium	400.000	90.000	70.000	70.000	70.000
Native aluminium	400.000	90.000	60.000	60.000	50.000
Painted aluminium	400.000	50.000	30.000	15.000	7.000
Enamelled steel	400.000	28.000	14.000	10.000	850
Painted iron	400.000	60.000	40.000	30.000	10.000
ABS polymer	400.000	70.000	60.000	60.000	60.000
Polystyrol	400.000	70.000	50.000	50.000	15.000
PTFE	400.000	90.000	90.000	70.000	50.000

N.B.: The figures are in thousand

3rd EXPERIMENT - DECONTAMINATION

Here too the results were virtually constant whichever detergent was used (soap and water; water and acetic acid; water and bicarbonate). The results of the 15 tests under each condition are averaged in table 5.

TABLE 5

SURFACE	DECONTAMINATION - 10^{-3} x no. cells at				
	0 ore	24 ore	48 ore	72 ore	144 ore
Stainless steel	900.000	5.000	5.000	5.000	4.000
Anodized aluminium	900.000	8.000	7.000	7.000	6.000

Native aluminium	900.000	9.000	9.000	9.000	9.000
Painted aluminium	900.000	5.000	3.000	3.000	3.000
Enamelled steel	900.000	1.000	700	600	600
Painted iron	900.000	7.000	5.000	5.000	5.000
ABS polymer	900.000	5.000	5.000	4.000	3.000
Polystyrol	900.000	4.000	4.000	4.000	4.000
PTFE	900.000	7.000	7.000	3.000	3.000

N.B.: The figures are in thousand

Comment

The following conclusions can be drawn from these results:

A) Spontaneous infection

On all the surfaces the bacterial level undergoes a fall which is apparent even at 24 hr. The greatest fall, which reached or exceeded 50% of the initial value, was on enamelled steel.

B) Provoked infection

No matter which organism was studied there was a rapid and significant inhibition of microbial growth. The greatest effect was on enamelled steel.

C) Decontamination

There was good decontamination at every surface no matter how infected or washed. Again this was most marked with enamelled steel.

It must be pointed out that the action of cold alone is sufficient to inhibit bacterial life independently of the material studied. However the constancy with which some surfaces show different degrees of bacterial inhibition compared with others leads to a consideration of a direct effect. It may readily be imagined that greater smoothness, greater compactness, a greater or less possibility of roughening or still worse of abrasions plays a role of first importance in decontamination. As far as spontaneous or provoked infection is concerned however it is necessary to invoke some chemical or physical factor which acts either directly on bacterial metabolism or by producing changes in the medium used (i.e. in the food or culture medium) and so lead to an inhibition of bacterial life.

Similar findings were made in a study of the mould flora which is often present in refrigerators and which is so difficult to eliminate once it becomes established. The degrees of inhibition which were found with bacterial cultures were closely followed with the moulds i.e.

1) Enamelled steel

maximum inhibition

2) Painted iron

medium inhibition

3) Painted aluminium

medium inhibition

4) ABS polymer

no inhibition

5) Polystyrol

no inhibition

6) Native aluminium

no inhibition

7) Stainless steel

no inhibition

8) Anodized aluminium

no inhibition

9) PTFE

no inhibition

The most significant part of this work is probably represented by these data taken together with those in the experimental part.