‘CLEAR SHIELD’

REPORT

On adherence of bacteria to surfaces treated with ‘Clear Shield’

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Introduction

'Clear-shield' is a surface treatment which forms a layer upon glass surfaces. Coated surfaces have been investigated, by other workers, for alkali attack, autoclavability, humidity/weathering, mechanical abrasion, and contact angles. All data indicate that the coating is a good protective agent for glass surfaces. Following these results it would appear that this treatment could be useful on glass surfaces to aid removal of contaminating micro-organisms from and could possibly hinder adhesion. Therefore an experiment was devised to study attachment and desportion of a strain of *Staphylococcus aureus*, a bacterium which has been frequently used in adherence work (Ashkenazi 1984; Jansson & Wadstrom 1984; Locci, Peters and Pulverer, 1981).

Methods

Bacterial suspension and growth conditions

A culture of *Staphylococcus aureus* NCTC4163 was inoculated into 100ml trypticase soya broth (TSB) in a conical flask. The starter culture was incubated at 37ºC on a rotary shaker at 120rpm for 18h after which 4ml was transferred to 100ml of fresh TSB and incubated in the same way for a further 18h. This culture was centrifuged for 15 min at 13,000g and the cell paste washed with phosphate buffed saline (PBS). The resultant suspension was centrifuged at 10,000g for 10 min and the cells again washed and resuspended in PBS. After a further wash procedure, the cells were finally suspended in 20ml PBS. Clusters of bacteria were dispersed by putting the suspension twice through a fine 25 gauge steel needle, followed by filtration through a glass microfibre filter paper (Whatman GF/A). The suspension was diluted with PBS to 450ml and 150ml dispensed into each of the 3 square trays with lids.

Slides

Thirty glass microscope slides were immersed overnight in a 2% solution of Decon 75. They were then thoroughly rinsed in running water for 15 mins before being dried. Both sides of the slide were cleaned with glass cleaner (Ritec). Of the thirty, ten were coated with ‘flat-glass Clear-Shield’, ten with ‘window-ware ClearShield’ and ten were left untreated as controls. These three groups of slides were each placed in one of the trays of bacterial suspension.

Bacterial adhesion and desorption

The trays containing the suspensions and glass microscope slides were incubated on a rotary shaker at 100rpm at 37ºC. After 3h, the slides were removed, washed with PBS and allowed to air dry. Five slides from each group were placed into individual boiling tubes containing 40ml of PBS, which were placed on a rotary shaker at 120rpm at 37º for one hour.

Bacterial counts

Of the 40ml, 0.2ml was directly plated-out, 1.0ml was used for a dilution series and 10ml was put through at 0.4µ Oxoid millipore filter. Both the washed and unwashed slides were stained with carbol fuchsin and counts were made of bacteria in 30 randomly selected fields of view on each slide.

Scanning Electron Microscopy (SEM)

Samples of coated and uncoated slides, prior to experimentation and after bacterial adhesion, were taken for observation with SEM. They were fixed in a vapour of 2% osmium tetroxide, coated with gold palladium in an Edwards 306 rotary coater (Edwards High Vacuum, Crawley, UK). Examinations were carried out with a 250 Stereoscan electron microscope (Cambridge Scientific Industries Ltd, Cambridge, UK) operated at 20Kv. Photographs were taken on 120 Kodak Tri-X film.

Results

The suspension of *Staphylococcus aureus* contained 2.8 x 10^9 cells. Immersion of the treated slides in the bacterial suspension was more difficult than the untreated slides as the coated surfaces appeared to be hydrophobic. However, the quantity of suspension in the tray was sufficient to give good constant coverage.

There was a noticeable macroscopic difference in the appearance of the non-treated and treated slides, the non-treated slides were considerably more opaque (Figure 1). This was entirely due to the number of cells adhering to the slides. Microscopically, counts were made of the one hundred and fifty fields of view on slides in each of the treatment groups. The results show a marked difference in adherence characteristics of the treated and non-treated slides (Table 1). The data on the counts of cells washed off the various slides as assessed by viable counts is given in Table 2.

Observations of typical fields of view are shown in Figures 2 – 4. There are obviously fewer cells adhering to the glass and window-coated slides (Figures 3 and 4) than to the non-treated control (Figure 2). There did appear to be slight differences between the glass and window coat. Fewer cells initially adhered to the glass-coated slides (Table 1) and the cells were slightly more concentrated than those adhering to the window-coated slides (Figure 3). However, in both these cases the removal, by washing, of significant numbers of a large proportion of the adherent cells was obvious (Tables 1 and 2; Figures 3 and 4).

There were slight discrepancies between the microscopic and viable wash-off counts, this can occur when comparing observed cells and plate-counts. The most noticeable differences were between the results of the percentage wash-off for the window and glass-coated slides. The figure for the wash-off viable count for the glass-coat treated slides in Table 2 being comparatively lower rather than that expected from the results in Table 1. In this case, the explanation probably lies in the fact that the group conformation of cells on the glass-coat treated slides makes counting extremely difficult.

The problem of adherence and wash-off as seen by the microscopic and viable counts was confirmed by scanning electron microscopy. Figure 5 shows a cleaned microscope slide. In Figures 6 and 7, the pattern of bacterial adhesion to the untreated slide is clearly demonstrated. However, it was extremely difficult to find any cells on the treated slides and the only ones seen are shown in Figures 8 and 9.

Table 1

Counts of cells adhering to slides prior to and post washing with PBS.

	Count of bacteria in one field of view (average 150 counts for each treatment group)		**% wash-off**
Treatment	**Cells adhering prior to washing**	**Cells reatined post washing**	
Window-ware ClearShield	115 ±39	26 ±18	77.4
Flat-glass ClearShield	76 ±36	25 ±29	67.2
Not treated	1641 ±470	800 ±167	51.3

Table 2

Average number of viable cells washed off from three groups of five slides.

Treatment	**Wash-off count (average per slide, x10^4)**
Window-ware ClearShield	3.6 ±1.1
Flat-glass ClearShield	7.0 ±4.3
Not treated	20 ±6.6

Discussion

The glass slides used were new, clean, with relatively scratch-free surfaces. Such surfaces are not usually thought to be as prone to bacterial attachment as those which are deliberately indented and marked, or are damaged by age or frequent handling. However, the ClearShield coatings clearly discouraged adsorption of *Staphylococcus aureus* to the relatively smooth glass surface (Table 1). There was at least a twenty-fold difference in adsorption of cells of *Staphylococcus aureus* to non-treated surfaces than to slides treated with 'glass coat ClearShield'. Numbers of bacteria on the grossly contaminated non-treatment slides were probably an underestimate caused by problems of counting cells which were closely adhering and lying on top of each other (Figures 6 and 7). In addition, considering the amount of material adhering to the non-treated surface, the wash-off from the coated slides was relatively much greater than that from non-treated surfaces.

In conclusion, the results clearly show that ClearShield coatings firstly impeded adherence of bacteria and secondly encouraged desportion by washing.

Figure 1 Non-treated and treated slides comparison

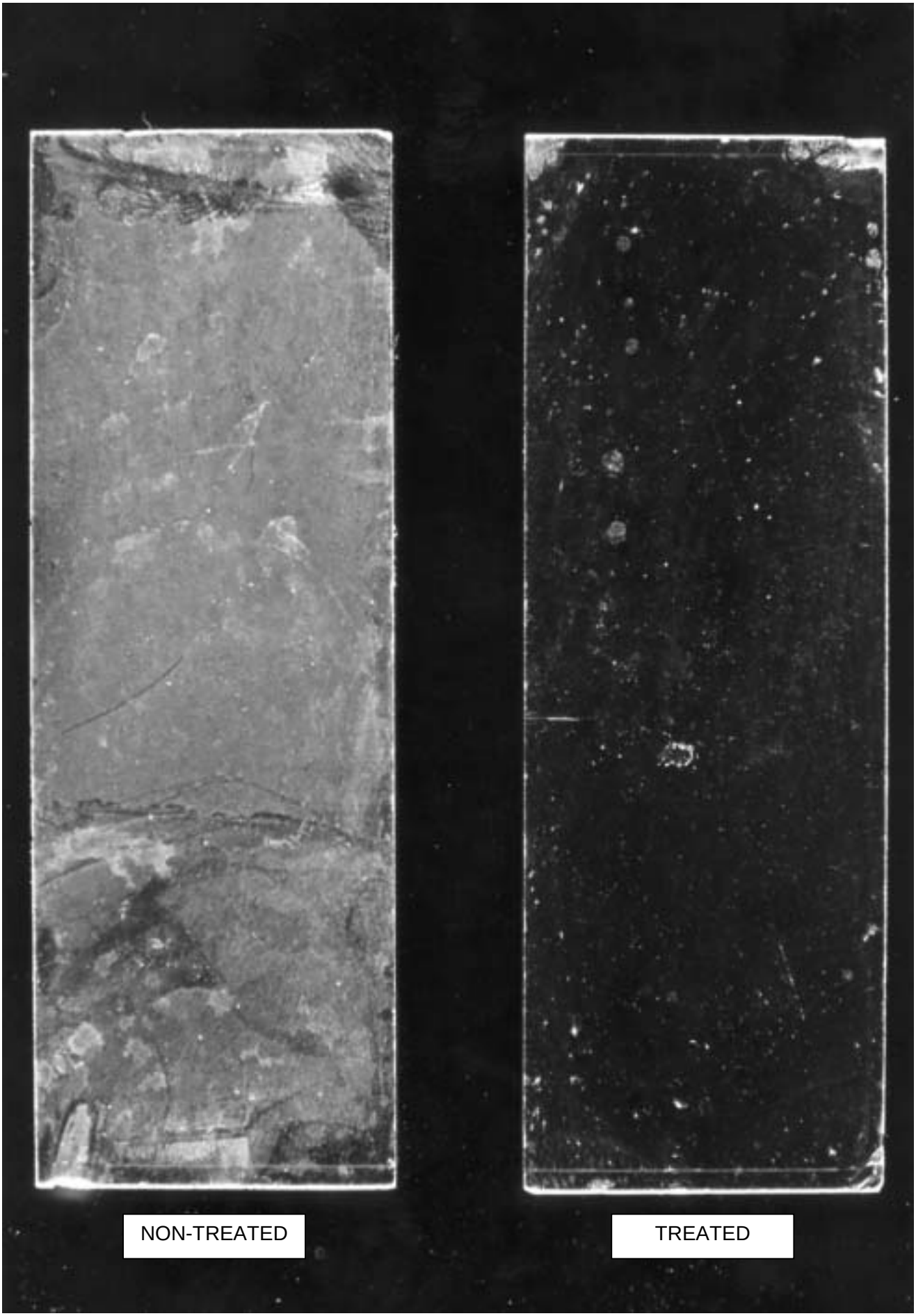
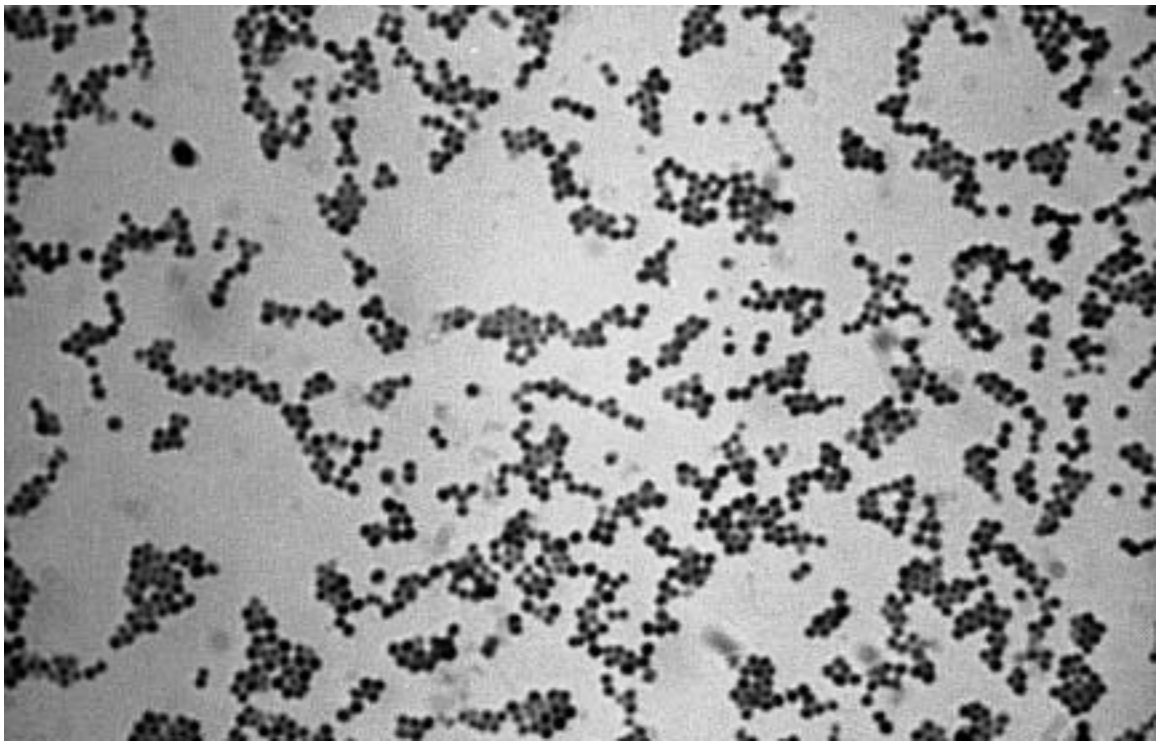
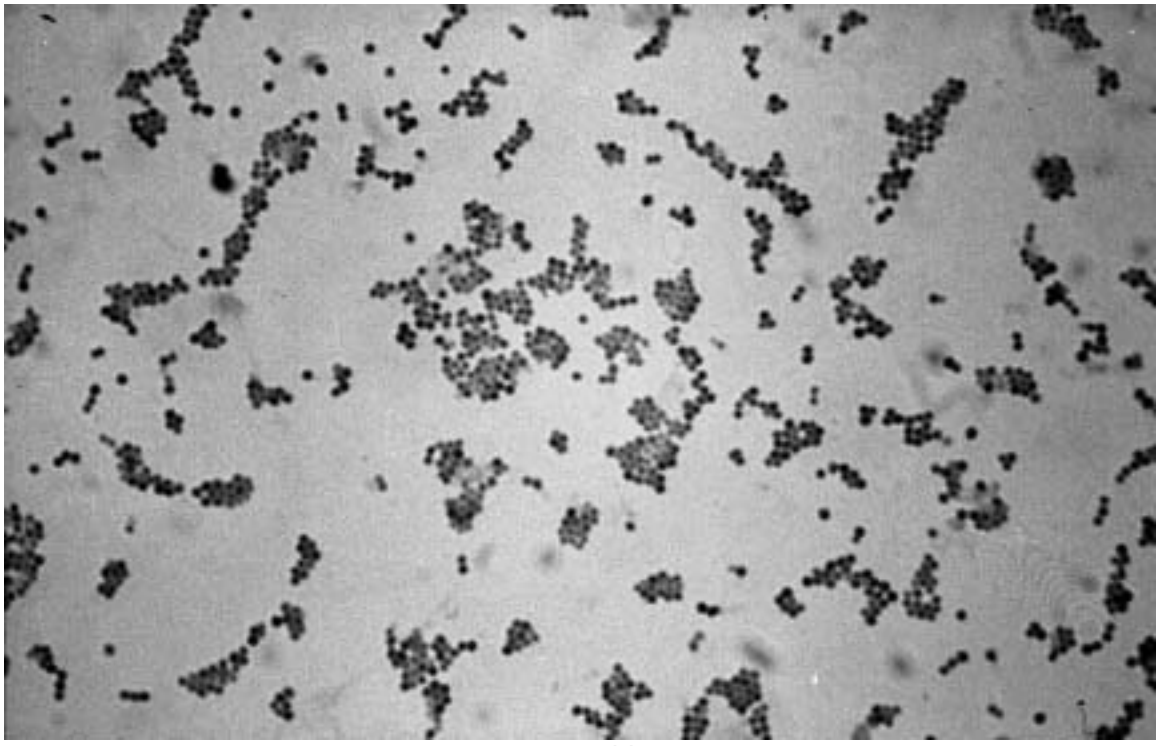


Figure 2 Micrograph of cells of *Staphylococcus aureus* adhering to glass slides

NON-TREATED



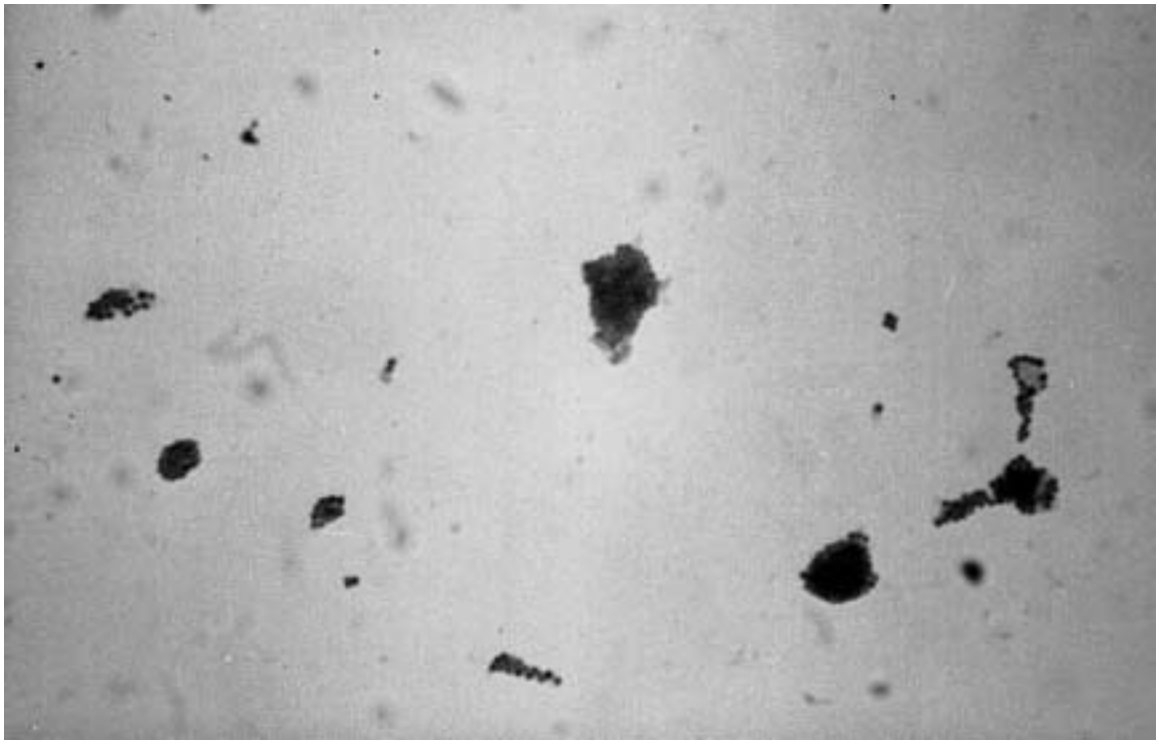
Prior to washing



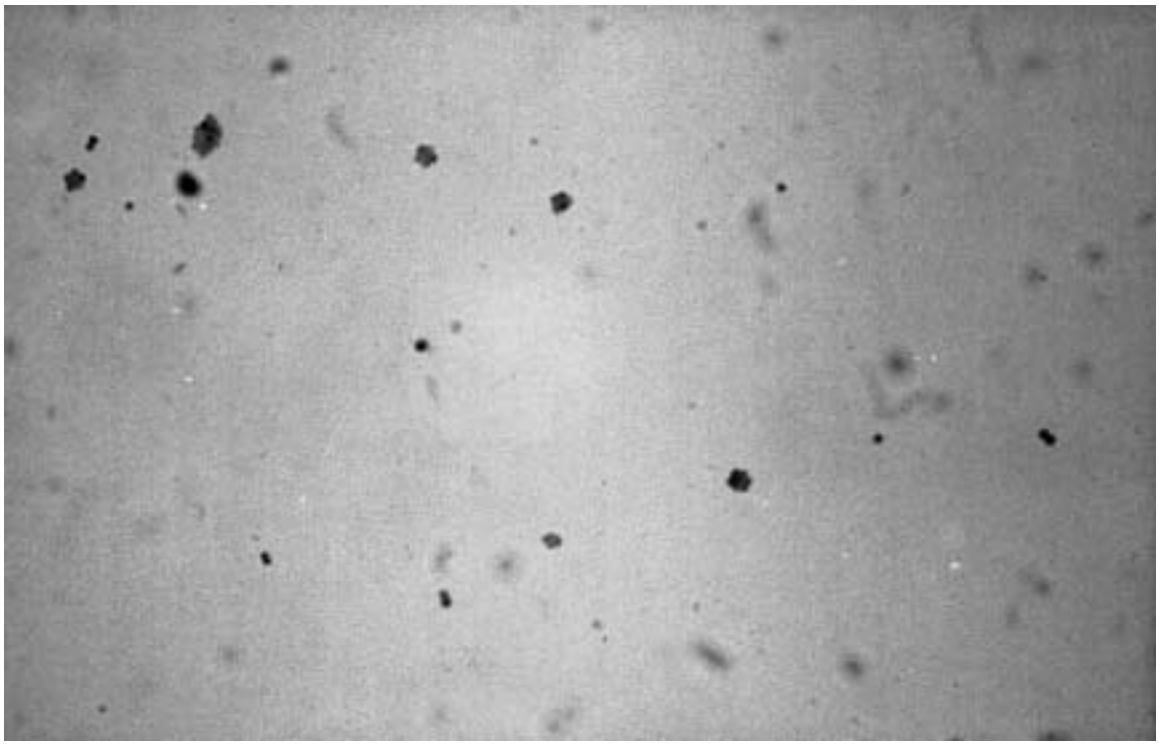
Post washing

Figure 3 Micrograph of cells of *Staphylococcus aureus* adhering to glass slides

GLASS-COAT



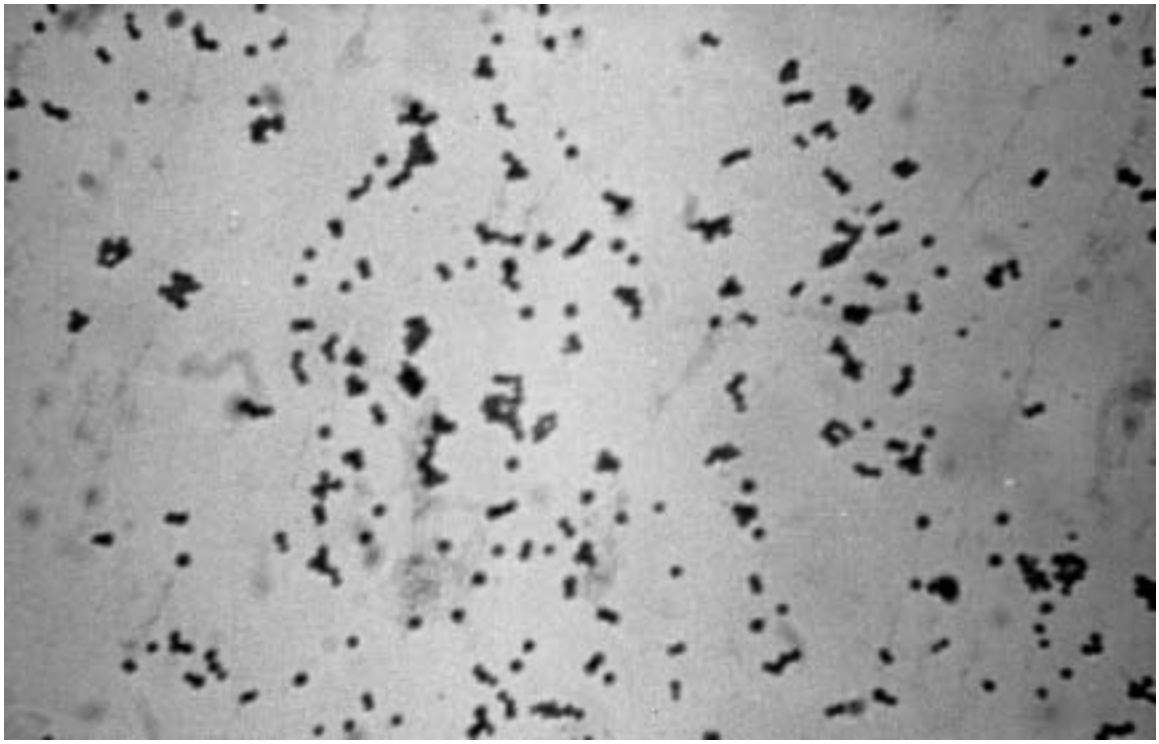
Prior to washing



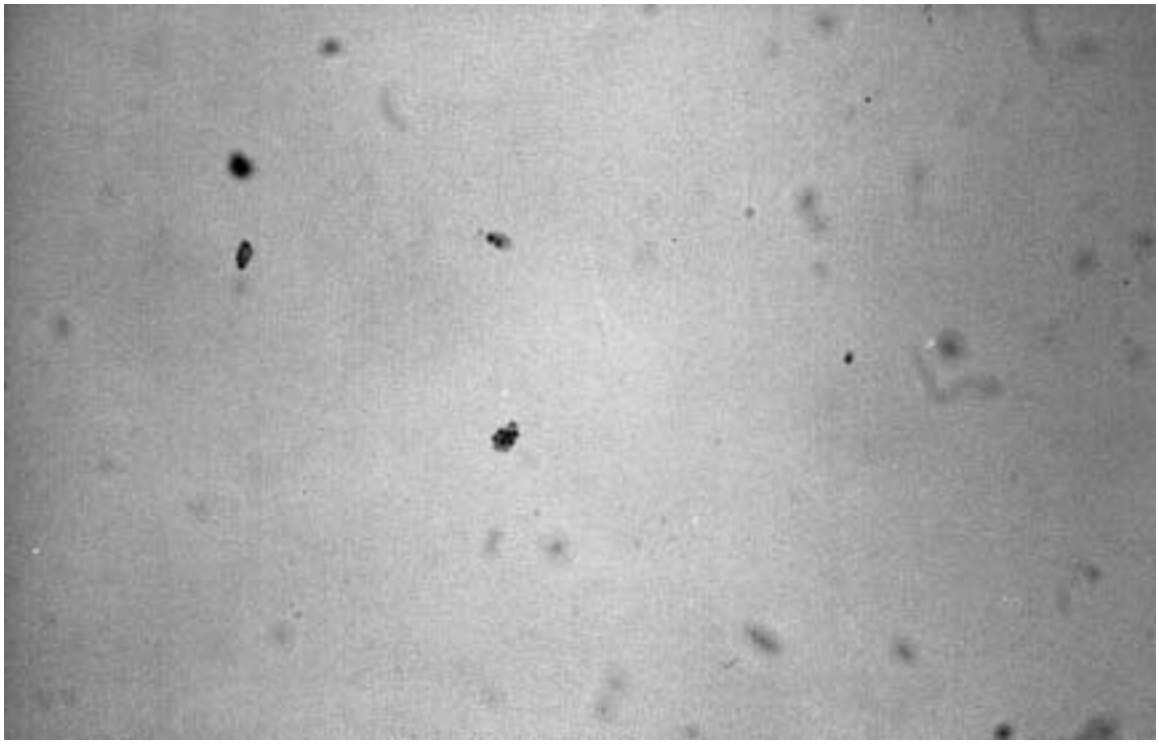
Post washing

Figure 4 Micrograph of cells of *Staphylococcus aureus* adhering to glass slides

WINDOW-COAT



Prior to washing



Post washing

Figure 5 Scanning electron micrograph of a clean microscope slide

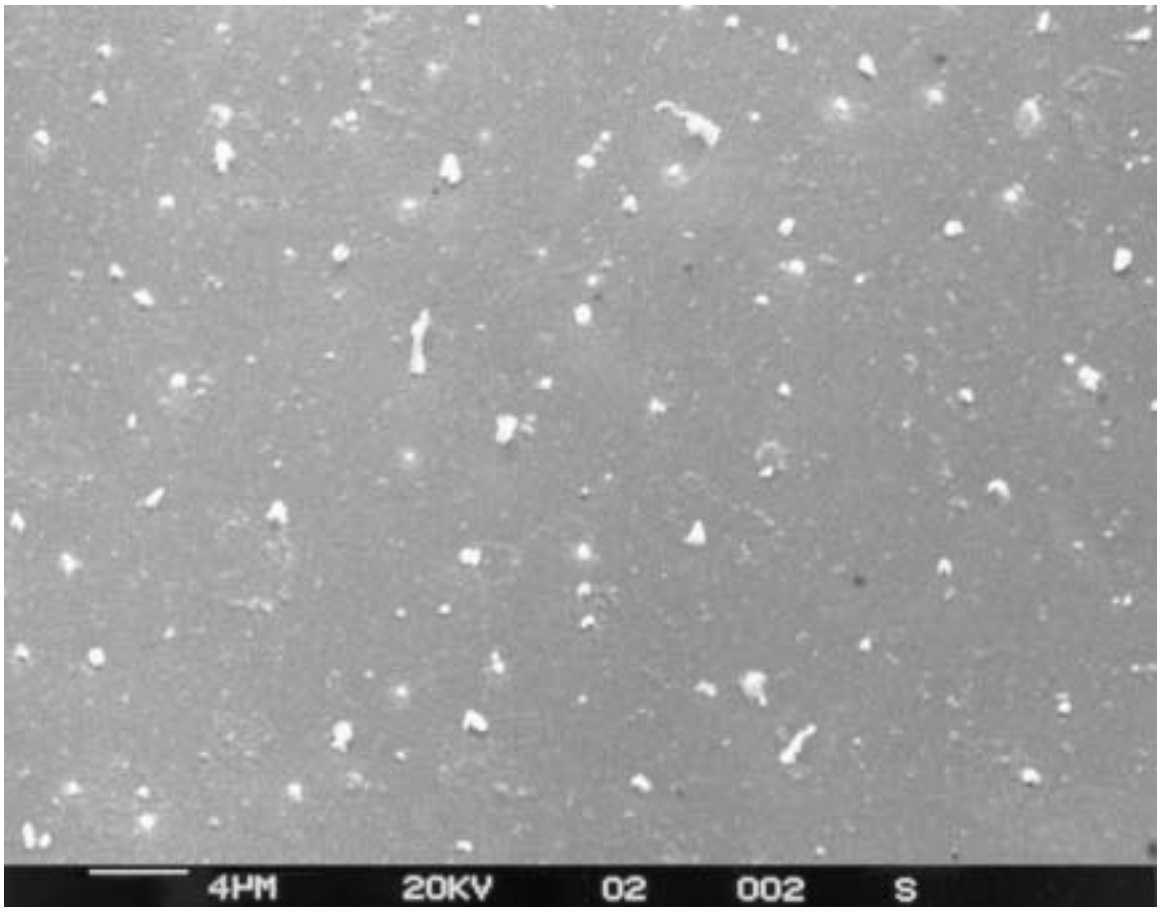


Figure 6 Scanning electron micrograph of cells of *Staphylococcus aureus* adhering to a clean non-treated microscope slide

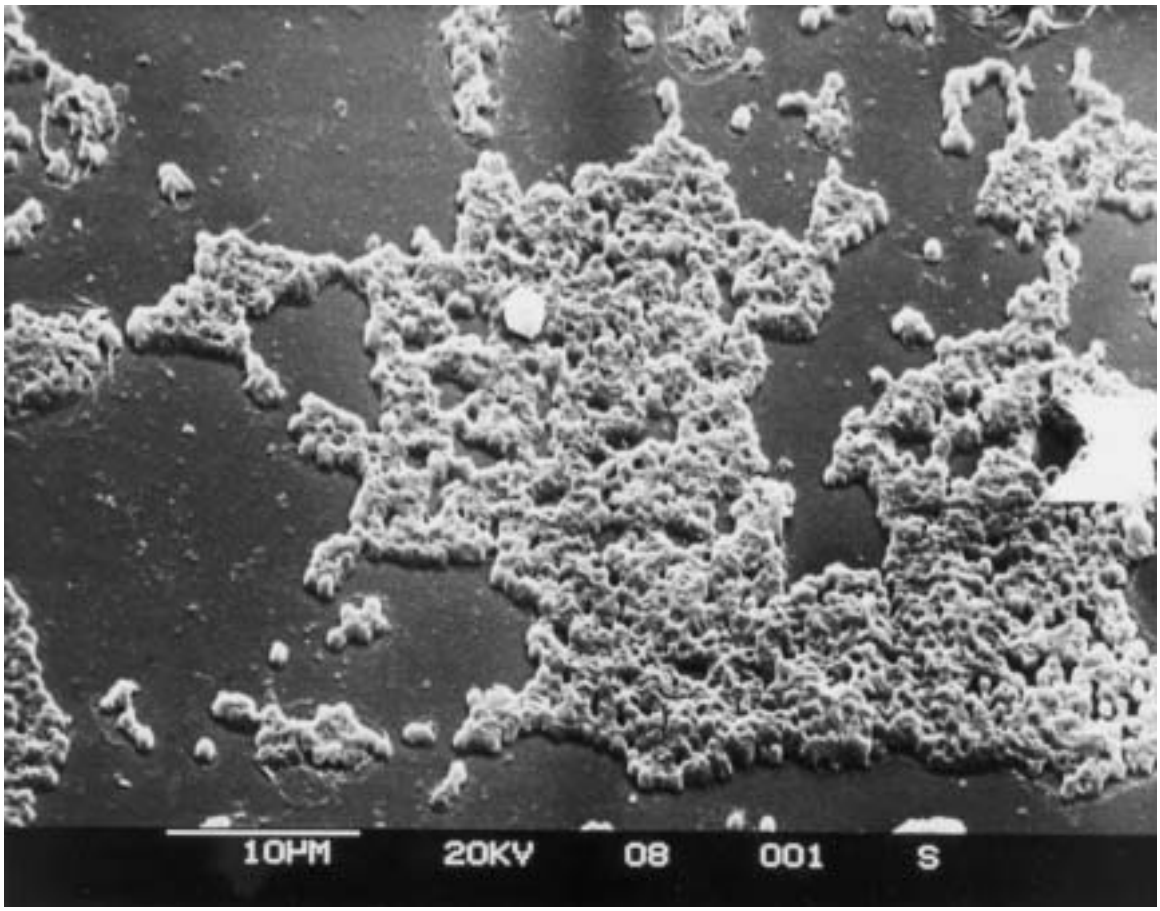


Figure 7 Scanning electron micrograph of cells of *Staphylococcus aureus* adhering to a clean non-treated microscope slide

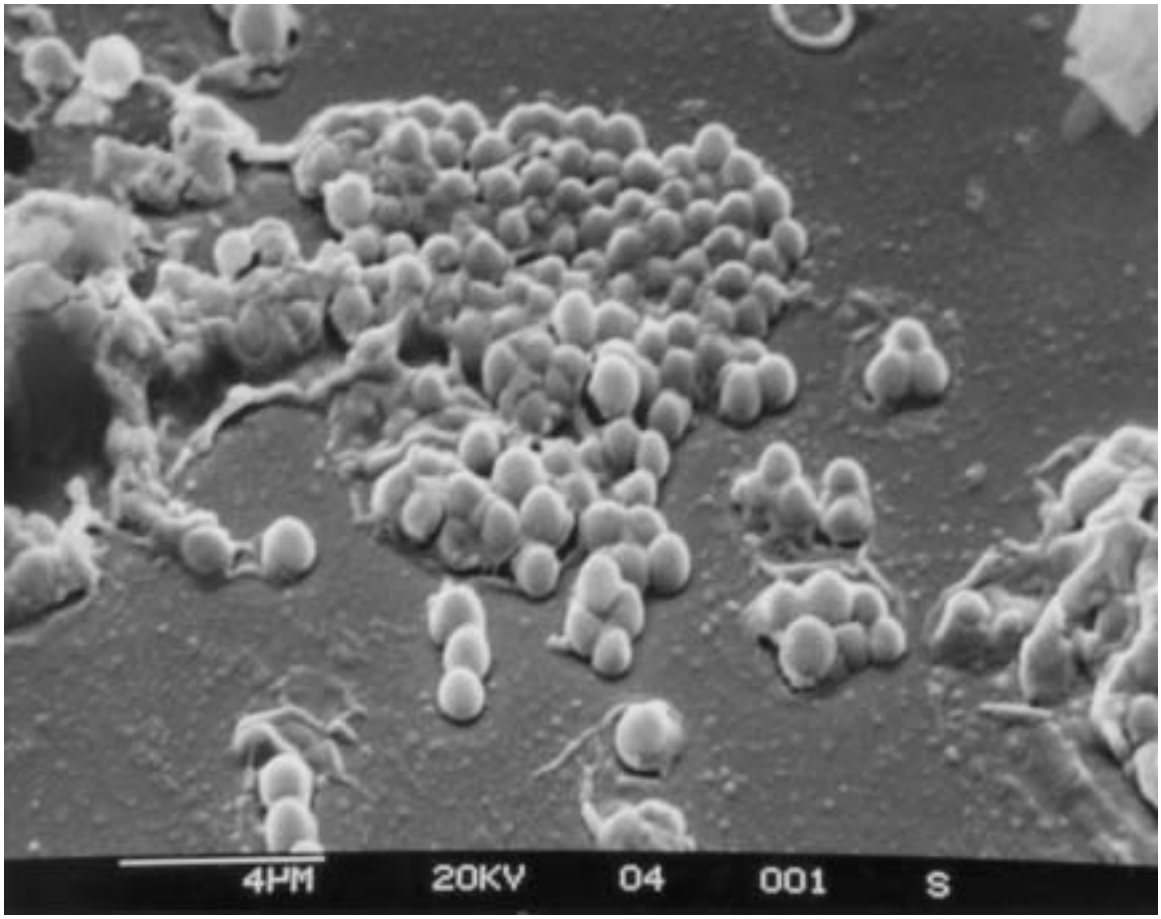


Figure 8 Scanning electron micrograph of cells of *Staphylococcus aureus* adhering to a window-coat treated microscope slide

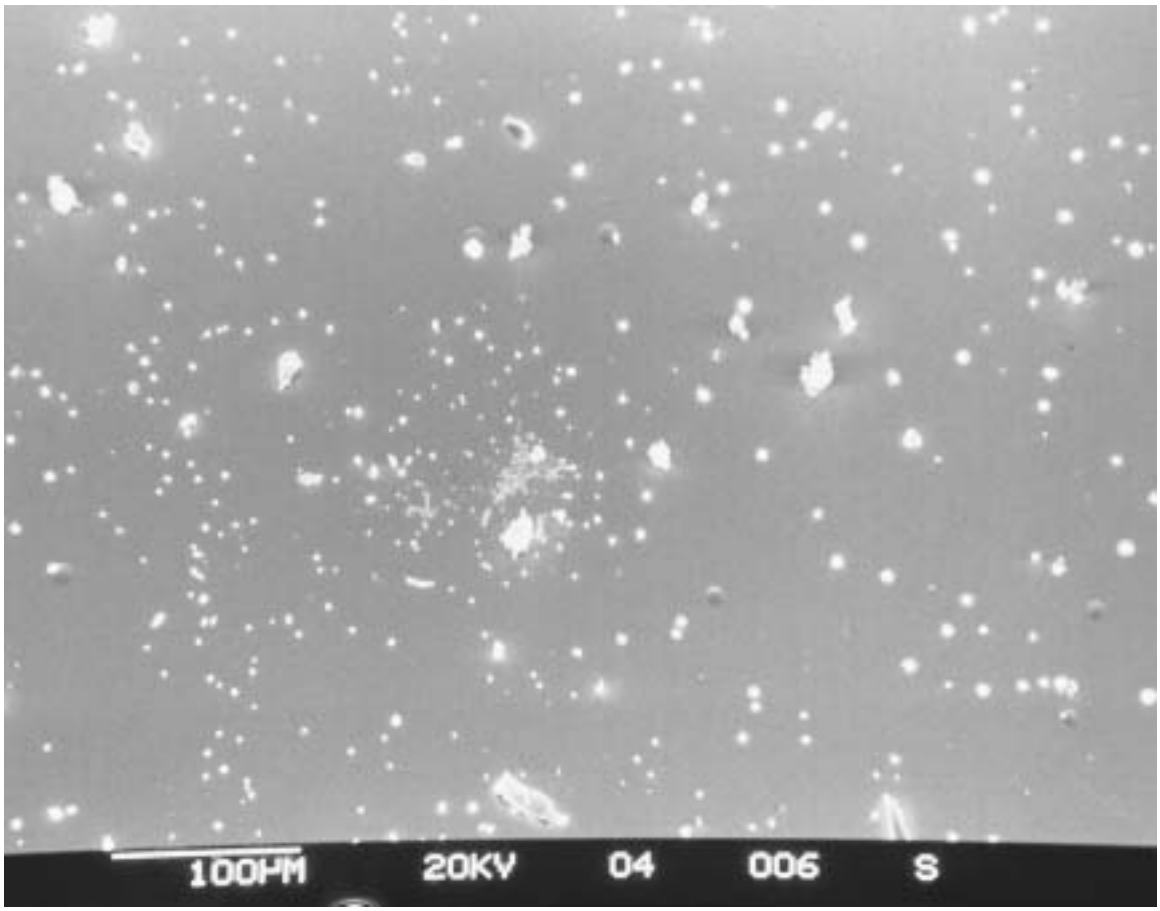


Figure 9 Scanning electron micrograph of cells of *Staphylococcus aureus* adhering to a window-coat treated microscope slide