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*Staphylococcus pseudintermedius* expresses surface proteins that closely resemble those  
from *Staphylococcus aureus*

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**Abstract**

*Staphylococcus pseudintermedius* is a commensal of dogs that is implicated in the pathogenesis of canine pyoderma. This study aimed to determine if *S. pseudintermedius* expresses surface proteins resembling those from *Staphylococcus aureus* and to characterise them. *S. pseudintermedius* strain 326 was shown to adhere strongly to purified fibrinogen, fibronectin and cytokeratin 10. It adhered to the  $\alpha$ -chain of fibrinogen which, along with binding to cytokeratin 10, is the hallmark of clumping factor B of *S. aureus*, a surface protein that is in part responsible for colonization of the human nares. Ligand-affinity blotting with cell-wall extracts demonstrated that *S. pseudintermedius* 326 expressed a cell-wall anchored fibronectin binding protein which recognised the N-terminal 29 kDa fragment. The ability to bind fibronectin is an important attribute of pathogenic *S. aureus* and is associated with the ability of *S. aureus* to colonize skin of human atopic dermatitis patients. *S. pseudintermedius* genomic DNA was probed with labelled DNA amplified from the serine-aspartate repeat encoding region of *clfA* of *S. aureus*. This probe hybridised to a single *SpeI* fragment of *S. pseudintermedius* DNA. In the cell wall extract of *S. pseudintermedius* 326 a 180 kDa protein was discovered which bound to fibrinogen by ligand-affinity blotting and reacted in a Western blot with antibodies raised against the serine-aspartate repeat region of ClfA and the B-repeats of SdrD of *S. aureus*. It is proposed that this is an Sdr protein with B-repeats that has an A domain that binds to fibrinogen. Whether it is the same protein that binds cytokeratin 10 is not clear.

## 1. Introduction

*Staphylococcus pseudintermedius* (until recently called *S. intermedius*) is a commensal of healthy dogs (Bannoehr *et al.*, 2007; Devriese *et al.*, 2005). It can also infect the skin of dogs suffering from atopic dermatitis causing pyoderma. The ability of *S. aureus* to adhere to desquamated epithelial cells is an important correlate of colonization of the nares of humans (Wertheim *et al.*, 2008). Clumping factor B (ClfB) and iron regulated surface determinant protein IsdA play important roles in adhesion to squamous cells and colonization of the nares of rodents, and in the case of ClfB, humans (Clarke *et al.*, 2006; Schaffer *et al.*, 2006; Wertheim *et al.*, 2008). ClfB binds to cytokeratin 10 (O'Brien *et al.*, 2002; Walsh *et al.*, 2004) which is expressed on the surface of squamous epithelial cells where it presumably provides a ligand for bacterial attachment. ClfB also binds to the  $\alpha$ -chain of fibrinogen in contrast to other fibrinogen binding surface proteins which bind to the  $\beta$ -chain or the  $\gamma$ -chain (Davis *et al.*, 2001; McDevitt *et al.*, 1997; Walsh *et al.*, 2008; Wann *et al.*, 2000). *S. pseudintermedius* adheres to canine corneocytes so it is reasonable to predict similar mechanisms of adhesion as for *S. aureus* (McEwan, 2000).

*S. pseudintermedius* adheres more strongly to corneocytes from regions of inflamed skin from a dog with atopic dermatitis than to non-inflamed areas suggesting that ligands for bacterial surface proteins are expressed at higher levels (McEwan *et al.*, 2006). In addition, fibronectin is present at elevated levels in the *stratum corneum* of atopic human skin whereas it was not detected in healthy skin (Cho *et al.*, 2001). This could provide a receptor for fibronectin binding proteins of *S. aureus*.

Given the similarity of *S. pseudintermedius* and *S. aureus* and the fact that both organisms adhere to squamous cells from their respective hosts as well as infect inflamed skin in atopic dermatitis it seems reasonable to expect that *S.*

*pseudintermedius* would display a repertoire of surface proteins similar to those of *S. aureus*. This study aimed to determine if *S. pseudintermedius* adheres to fibrinogen, fibronectin, elastin and cytokeratin 10 and to characterize the surface proteins responsible.

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## 2. Materials and Methods

### 2.1. Bacterial strains and culture conditions.

*S. pseudintermedius* strains used in this study were isolates from cases of canine pyoderma and were a kind gift from Dr. Neil McEwan, University of Liverpool. *S. aureus* strain Newman is a human clinical isolate (Duthie and Lorenz, 1952), Newman *clfB* is a mutant deficient in clumping factor B (Ni Eidhin *et al.*, 1998) and Newman *clfA clfB* lacks clumping factor A and ClfB (Fitzgerald *et al.*, 2006). *S. aureus* SH1000 shows strong adherence to extracellular matrix proteins and is a derivative of strain 8325-4 with a repaired defect in *rsbU* (Horsburgh *et al.*, 2002). *S. aureus* P1 adheres strongly to fibronectin (Fitzgerald *et al.*, 2006; Roche *et al.*, 2004; Sherertz *et al.*, 1993).

Strains were grown in brain heart infusion (BHI, Oxoid) broth at 37°C with aeration. Stationary phase cultures were grown for approximately 16 hours. Exponential phase cultures were inoculated 1:100 from overnight starter cultures. Cells were washed in BHI and grown to an optical density of 0.6.

### 2.2. Ligand and Western immunoblot analysis.

Exponential or stationary phase cultures were harvested, washed in phosphate-buffered saline (PBS) and resuspended to OD<sub>600</sub> of 40 in lysis buffer (50 mM Tris/HCl, 20 mM MgCl<sub>2</sub>, pH 7.5) supplemented with 30 % (w/v) raffinose and complete protease inhibitors (40 µl/ml, Roche). Cell wall proteins were solubilised by incubation with lysostaphin (200 µg/ml; AMBI, New York) for 10 min at 37°C. Protoplasts were removed by centrifugation at 12000 *g* for 10 min and the supernatant containing solubilised cell wall proteins was aspirated and boiled for 5 min in final sample buffer (0.125 M Tris/HCl, 4 %, w/v, SDS, 20 % glycerol, 10 %, v/v, 2-mercaptoethanol, 0.002

%, w/v, bromphenol blue). Proteins were separated on 7.5 % (w/v) polyacrylamide gels and electrophoretically transferred onto PVDF membranes (Roche) and blocked in 10 % (w/v) skimmed milk (Marvel).

Blots were probed with anti-ClfA SD-repeat antibodies (1:1,000; a gift from O. Hartford, Trinity College Dublin), anti-SdrD B-repeat antibodies (1:1,000; a gift from L. O'Brien, Trinity College Dublin) or fibrinogen (20 µg/ml, Calbiochem). Bound antibodies were detected using horseradish peroxidase-conjugated (HRP) protein A (1:500; Sigma) and bound fibrinogen was detected with HRP-conjugated anti-fibrinogen antibody (1:3,000, Dako). Biotinylated fibronectin was used in ligand-affinity blots. Human fibronectin (0.5 mg/ml in PBS) was incubated with biotin (2mg/ml) for 20 min at room temperature. The reaction was stopped by addition of 10 mM NH<sub>4</sub>Cl. Excess biotin was removed by dialysis against PBS overnight at 4 °C. Blots were probed with biotinylated fibronectin and POD-conjugated streptavidin (1:5000; Roche). Reactive bands were visualised using the LumiGLO Reagent and peroxide detection system (Cell Signalling Technology).

Filters to be reprobed with another antibody were stripped using a solution of 2% w/v SDS, 100 mM β-mercaptoethanol and 50 mM Tris at 50 °C for 30 min, washed twice for 10 min in TS (10 mM Tris-HCl, 0.9 % (w/v) NaCl, pH 7.4) buffer and then blocked in 10 % Marvel for 2 – 18 h.

### 2.3. Bacterial adherence to fibrinogen and fibronectin

Microtitre plates (Sarstedt) were coated with doubling dilutions of human fibrinogen (Calbiochem), canine fibrinogen (Sigma) or human fibronectin (Calbiochem) in PBS. Plates were coated overnight at 4°C and blocked for 2 h at 37°C with 5 % (w/v) bovine serum albumin (BSA). Washed exponential or stationary phase cells were



adjusted to an OD<sub>600</sub> of 1.0 in PBS, and 100 µl was added to each well and incubated for 2 h at 37°C. After washing with PBS, adherent cells were fixed with formaldehyde (25% v/v), stained with crystal violet and the A<sub>570</sub> measured. Adherence assays with recombinant cytokeratin 10, recombinant fibrinogen α-chain (both gifts from H. Miajlovic, Trinity College Dublin) and purified fibronectin N29 fragment were performed with Nunc microtitre plates using sodium carbonate buffer (pH 9.6) instead of PBS.

#### *2.4. Bacterial adhesion to immobilized elastin peptides*

Bacterial adhesion to immobilized elastin peptides was performed as previously described (Roche *et al.*, 2004). Briefly, microtitre plate wells (Povair) were coated with doubling dilutions aortic elastin peptides (Elastin Products Company) in PBS and air dried under UV light (366 nm) at room temperature for 18 h. Wells were blocked for 2 h at 37°C with 5% BSA. Bacteria were washed with PBS and resuspended to an OD = 1.0 ( $1 \times 10^8$  colony forming units ml<sup>-1</sup>). Bacterial cell adherence was measured using a fluorescent nucleic acid stain SYTO-13 (Molecular Probes). Bacterial cells were incubated with SYTO-13 (5 µM) at room temperature for 15 min in the dark. Elastin-coated wells were washed three times with PBS and 100 µl of stained cells was added to the plate and incubated with shaking in the dark for 1 h. Wells were washed three times with PBS and adherent bacteria were measured using an LS-50B spectrophotometer (Perkin-Elmer) with excitation at 488 nm and emission at 509 nm.

#### *2.5. Inhibition of bacterial adherence to immobilised fibronectin with fibronectin fragments*

The N29 fragment, the gelatin-binding fragment and the 110-kDa cell-binding region of fibronectin were isolated following the protocol reported by Borsi *et al.* (1986). The purity of the fragments was assessed by SDS-PAGE.

Microtitre plates were coated with fibronectin (10 µg/ml) in PBS overnight at 4°C and blocked for 2 h at 37°C with 5 % (w/v) BSA. *S. pseudintermedius* was incubated with increasing concentrations of fibronectin fragments for 1 h at 37°C before being added to the wells of a fibronectin coated plate. After washing with PBS, adherent cells were fixed with formaldehyde (25% v/v), stained with crystal violet and the A<sub>570</sub> measured.

## 2.6. Southern Hybridisation

Genomic DNA was isolated from *S. pseudintermedius* strain 326 using the Genomic DNA purification kit from Edge BioSystems. *S. pseudintermedius* cells were treated with 200 µg lysostaphin (AMBI, New York) for 10 min at 37°C to digest the cell-wall peptidoglycan and the remainder of the procedure was carried out according to the manufacturer's instructions. Genomic DNA was digested with *SpeI* for 16 h at 37°C before separation on 1 % (w/v) agarose gel. DNA was depurinated, fragmented, denatured and transferred to positively charged membranes (Roche) by capillary transfer in 20 x SSC (3 M NaCl, 0.3 M NaCitrate) and immobilised by baking at 120°C for 2 h. Prehybridisation and hybridization were carried out at a low temperature (52°C) for a reduced stringency Southern blot. The membrane was incubated in a standard prehybridization solution (5 x SSC, 0.1% N-laurylsarcosine, 0.02% SDS, 1 x Blocking reagent (Roche)) for 3 hours at 52°C and then hybridized for 16 hours in the same solution containing 0.5 µg/ml DIG-labelled DNA probe. DIG-labelled probes were synthesised by PCR using the plasmid pCF77 (Hartford *et al.*, 1997) as template,

primers SDF (5'-TCAGATTCAGCGAGTGATTC-3') and SDR (5'-GAATCACTTGATGAATCGG-3') and DIG-dNTPs. Membranes were washed and developed according to the instructions of the DIG system, using CPD-star (Roche) as the chemiluminescent substrate. Membranes were exposed to X-ray film (X-Omat, Kodak) and developed.

### 3. Results

#### 3.1. Adherence of *S. pseudintermedius* strains to fibrinogen, fibronectin and cytokeatin 10.

Eight *S. pseudintermedius* clinical isolates from cases of canine pyoderma were grown to mid exponential phase and tested for adherence to human fibrinogen, fibronectin and cytokeatin 10. *S. aureus* strain SH1000 was used as a reference strain because it adheres strongly to fibronectin and cytokeatin 10 in the exponential phase of growth and to fibrinogen in both exponential and stationary phases. *S. aureus* Newman does not express fibronectin binding proteins (Grundmeier *et al.*, 2004). A mutant of *S. aureus* Newman lacking ClfA and ClfB was used as a negative control in adhesion assays. These data show that *S. pseudintermedius* strains grown to the exponential growth phase can adhere to immobilised human fibrinogen, fibronectin and cytokeatin 10 but to varying degrees (Fig. 1). Strong adherence to one ligand did not necessarily correlate with strong binding to the other ligands.

#### 3.2. Adherence of *S. pseudintermedius* strain 326 to fibrinogen, fibronectin, cytokeatin 10 and elastin.

*S. pseudintermedius* strain 326 was chosen for further investigation because it adhered strongly to the ligands tested and a more detailed analysis was performed in

comparison to *S. aureus* SH1000. *S. pseudintermedius* adhered strongly to immobilized human cytokeratin 10 and fibronectin in a dose-dependent and saturable manner (Fig. 2). Adherence was higher for bacteria from the exponential phase of growth whereas adherence to fibrinogen was comparable in the exponential and stationary phases (Fig. 2). This is a very similar trend to that seen for *S. aureus* SH1000. *S. pseudintermedius* 326 and *S. aureus* SH1000 adhered to human fibrinogen (Fig. 2) and canine fibrinogen (data not shown) with similar affinities. *S. pseudintermedius* also adhered to elastin, a phenotype which is the hallmark of fibronectin binding proteins of *S. aureus* (Roche *et al.*, 2004). It can be concluded that *S. pseudintermedius* seems to express ligand binding activities that resemble those promoted by clumping factors and fibronectin binding proteins of *S. aureus*

### 3.3. Adherence of *S. pseudintermedius* to recombinant fibrinogen $\alpha$ -chain.

*S. pseudintermedius* strain 326 was assessed for its ability to adhere to the purified recombinant  $\alpha$ -chain of human fibrinogen. The *S. aureus* surface protein ClfB mediates adherence to the fibrinogen  $\alpha$ -chain (Walsh *et al.*, 2008). An isogenic *clfB* mutant of strain Newman (Newman *clfB*) was used as a control. *S. pseudintermedius* 326 and *S. aureus* strain Newman grown to the exponential phase adhered to immobilised  $\alpha$ -chain in a dose-dependent and saturable manner while a ClfB-defective mutant of Newman did not (Fig. 3). Thus *S. pseudintermedius* adheres strongly to the  $\alpha$ -chain of fibrinogen suggesting that it expresses a surface protein related to ClfB of *S. aureus*. The ability of *S. pseudintermedius* to adhere to the  $\beta$ -chain or  $\gamma$ -chain of fibrinogen was not tested.

### 3.4. Inhibition of adherence of *S. pseudintermedius* to fibronectin by fibronectin fragments.

To identify the region of fibronectin recognised by *S. pseudintermedius*, fragments of fibronectin were tested for their ability to inhibit adhesion of *S. pseudintermedius* to the immobilized ligand. The N-terminal 29 kDa (N29) fragment, a 42 kDa gelatin binding fragment and 110kDa cell-binding fragment obtained by digestion of human fibronectin with thermolysin were preincubated with *S. pseudintermedius* before being added to the wells of a fibronectin-coated microtitre dish. Only the N29 domain inhibited adherence of *S. pseudintermedius* to immobilised fibronectin. Complete inhibition was achieved at the highest concentration tested (Fig. 4A). *S. pseudintermedius* also adhered directly to immobilised N29 in a dose-dependent manner (Fig. 4B). This implies that *S. pseudintermedius* specifically recognises the N-terminal region of fibronectin, the same region recognised by FnBPA and FnBPB of *S. aureus*.

### 3.5. Western and ligand affinity blotting with cell-wall extracts of *S. pseudintermedius*.

In order to determine if *S. pseudintermedius* expressed a cell wall-associated protein with serine-aspartate repeats, cell wall extracts were prepared from stabilized protoplasts, separated by SDS-PAGE gel electrophoresis and evaluated by Western immunoblotting with antibodies raised against the serine-aspartate dipeptide repeated region of ClfA from *S. aureus* (data not shown). The presence of a protein of 180 kDa in the solubilized cell wall fraction implies that a protein with SD repeats homologous to ClfA-Sdr proteins is anchored to the cell wall surface. The filter was stripped of bound antibody and re-probed with an antibody raised against the B-repeats of SdrD

from *S. aureus*. A 180 kDa protein also reacted with anti-B repeat antibody (data not shown). This raised the possibility that the same protein from *S. pseudintermedius* may possess B repeats and SD repeats like SdrC, SdrD and SdrE of *S. aureus*.

The filter was then stripped and reprobed with human fibrinogen followed by HRP-conjugated anti-fibrinogen antibody. This also recognized a protein of 180 kDa (data not shown) suggesting that the same protein may be reacting with anti-SD repeat and anti-B repeat antibodies and also fibrinogen.

### 3.6. Fibronectin affinity blotting with cell wall extracts from *S. aureus* and *S. pseudintermedius*.

Solubilized cell wall-associated proteins were examined by fibronectin-affinity blotting. *S. aureus* strains P1 and SH1000 expressed proteins with apparent molecular masses of 180 kDa that reacted with fibronectin (Fig. 5). FnBPA and FnBPB of 8325-4 (and its derivative SH1000) are almost identical in size and run as a doublet band in SDS-PAGE gels. Cell wall protein extracts from *S. pseudintermedius* 326 contained a protein band of 130 kDa that reacted with fibronectin (Fig. 5). There is evidence of some degradation in each of the samples. It appears that *S. pseudintermedius* expresses a cell-wall anchored fibronectin binding protein that is 50 kDa smaller than FnBPA and FnBPB of *S. aureus* SH1000.

### 3.7. Southern hybridisation with an SD-repeat probe

Cell-wall extracts from *S. pseudintermedius* reacted with an anti-SD repeat antibody raised against ClfA of *S. aureus*. This strongly suggests that *S. pseudintermedius* expresses a protein with SD repeats. To investigate this further, Southern hybridisation of *S. pseudintermedius* genomic DNA was performed with a

DNA probe corresponding to the SD repeat-encoding region of *clfA* from *S. aureus*. *S. pseudintermedius* genomic DNA was digested with the restriction enzyme *SpeI*. Southern hybridisation under conditions of low stringency revealed a single *SpeI* fragment of approximately 6.2 kb which hybridised with the probe (Fig. 6). When *Sau3A* or *ClaI* digests were analyzed, a single fragment of 2 kb hybridised with the probe (data not shown) indicating that there is likely to be only one gene with DNA encoding SD-repeats in the *S. pseudintermedius* genome.

#### 4. Discussion

The pathogenesis of canine atopic dermatitis is poorly understood. Enterotoxins expressed by *S. pseudintermedius* have been identified as potential virulence factors in the progression of the disease (Hendricks *et al.*, 2002). The ability of *S. pseudintermedius* to adhere to canine corneocytes is likely to be an important factor in initiation of infection. However, the factors involved in adherence to and colonisation of healthy or damaged canine skin have not been identified. In *S. aureus*, cell wall-associated surface proteins mediate bacterial adherence to human desquamated nasal epithelial cells. It is likely that *S. aureus* surface proteins are also involved in adherence to the skin of patients suffering from atopic dermatitis. This study aimed to identify protein ligands for *S. pseudintermedius adhesins* and also to determine if *S. pseudintermedius* expressed surface proteins similar to those from *S. aureus*.

Several *S. pseudintermedius* isolates from cases of canine pyoderma adhered to fibrinogen, fibronectin and cytokeratin 10. *S. pseudintermedius* strain 326 showed high levels of binding to all three ligands and was chosen for further investigation. *S. pseudintermedius* 326 adhered to the  $\alpha$ -chain of human fibrinogen. In *S. aureus*, ClfB mediates binding to the fibrinogen  $\alpha$ -chain (Walsh *et al.*, 2008). The  $\alpha$ -chain binds in a

trench separating the N2 and N3 subdomains probably by the dock, lock and latch mechanism (Ponnuraj *et al.*, 2003). ClfB also binds to cytokeratin 10 (Walsh *et al.*, 2004). Since binding to these two ligands is mediated exclusively by ClfB in *S. aureus* it is possible that *S. pseudintermedius* encodes a homologue of that protein. Adherence of *S. pseudintermedius* to the fibrinogen  $\gamma$ - or  $\beta$ -chains was not tested here.

*S. pseudintermedius* also expresses a fibronectin binding protein that recognizes the N-terminal 29 kDa domain. This region is comprised of F1 modules that bind to *S. aureus* FnBPA and FnBPB by a tandem  $\beta$ -zipper mechanism (Pilka *et al.*, 2006; Schwarz-Linek *et al.*, 2003). The fibronectin binding protein of *S. pseudintermedius* appeared to be smaller than FnBPs from *S. aureus* P1 and SH1000. One reason for the size difference could be that the *S. pseudintermedius* protein possesses fewer fibronectin binding repeats. It has been noted previously that FnBPA proteins can contain more or less fibronectin binding repeats than that of 8325-4 (Rice *et al.*, 2001). Alternatively, the fibronectin binding protein from *S. pseudintermedius* could have undergone proteolytic degradation either on the bacterial cell surface during growth or during the lysostaphin digestion and preparation of cell wall extracts.

The A domains of FnBPA and FnBPB of *S. aureus* bind to fibrinogen and elastin (Keane *et al.*, 2007). At least seven different isotypes of FnBPA from *S. aureus* exist and all bind to fibrinogen and elastin with similar affinity despite considerable antigenic differences (Loughman *et al.*, 2008). *S. pseudintermedius* also binds to elastin but whether this elastin binding activity is mediated by a fibronectin binding protein is not clear.

An antibody raised against the SD-repeats of ClfA from *S. aureus* reacted with a 180 kDa cell wall-anchored protein of *S. pseudintermedius*. A cell wall-anchored protein of the same size also reacted with anti-SdrD B repeat antibodies and also bound



to fibrinogen in a ligand-affinity blot. Although it is possible that two or three distinct similar sized proteins occur, it is more likely that *S. pseudintermedius* expresses one protein with B-repeats and SD repeats that also binds to fibrinogen. The SdrG protein of *S. epidermidis* has these properties and specifically binds to the B $\beta$ -chain of fibrinogen. The binding of *S. pseudintermedius* to the B $\beta$ -chain was not tested here. In *S. aureus*, surface proteins with very similar A domains (ClfA, ClfB, SdrG, FnBPA and FnBPB) have distinct C-terminal domains (SD repeats, B repeats, fibronectin binding repeats). It is possible that surface proteins of *S. pseudintermedius* could represent novel combinations of A domains and C-terminal domains.

It is possible that *S. pseudintermedius* expresses iron-regulated surface proteins that are expressed when bacteria are grown in iron-limiting conditions. The iron-regulated surface protein IsdA plays a role in adherence of *S. aureus* to desquamated nasal epithelial cells and confers resistance to bactericidal lipids in skin and to the bactericidal effect of lactoferrin (Clarke and Foster, 2008; Clarke *et al.*, 2007; Clarke *et al.*, 2004). *S. pseudintermedius* may express a homologue of IsdA that promotes adherence to canine corneocytes and survival on canine skin. Assays should be performed with *S. pseudintermedius* grown in iron-limiting media and cell-wall extracts could be tested for the presence of proteins only expressed under iron-limiting conditions.

It is likely that surface proteins of *S. pseudintermedius* are virulence determinants in canine pyoderma and play a role in adherence to canine corneocytes. Cloning the genes and characterisation of the surface proteins would allow them to be assessed as candidate antigens for vaccination against canine pyoderma. Determining the genomic DNA sequence of at least one isolate from canine pyoderma would identify

putative virulence factors of *S. pseudintermedius* and facilitate design of vaccines to combat the disease.

### **Acknowledgements**

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**Figure Legends**

**Fig. 1 Adherence of *S. aureus* SH1000 and *S. pseudintermedius* strains to immobilised ligands.** Plates were coated with human fibronectin (A), cytokeratin 10 (B) or human fibrinogen (C) at 5  $\mu\text{g/ml}$ . *S. aureus* SH1000, *S. aureus* Newman *clfA* *clfB* or *S. pseudintermedius* strains (numbered) were added. Results shown are the mean values of triplicate samples. Error bars show the standard deviation.

**Fig. 2 Adherence of *S. aureus* and *S. pseudintermedius* to immobilised ligands.** Plates were coated with doubling dilutions of fibronectin (A), cytokeratin 10 (B), human fibrinogen (C) or elastin (D). *S. pseudintermedius* 326 from exponential ( $\circ$ ) or stationary phase ( $\bullet$ ), *S. aureus* SH1000 cells from exponential ( $\square$ ) or stationary phase ( $\blacksquare$ ), *S. aureus* P1 cells from exponential phase ( $\blacktriangle$ ) or Newman *clfA* *clfB* from exponential phase ( $\nabla$ ) were added. Results shown are the mean values of triplicate samples. Error bars show the standard deviation.

**Fig. 3 Adherence of *S. pseudintermedius*, *S. aureus* Newman and *S. aureus* Newman *clfB* to immobilised fibrinogen  $\alpha$ -chain.** Plates were coated with doubling dilutions of fibrinogen  $\alpha$ -chain. *S. pseudintermedius* 326 ( $\circ$ ), *S. aureus* Newman ( $\bullet$ ) and *S. aureus* Newman *clfB* ( $\blacksquare$ ) were added. Results shown are the mean values of triplicate samples. Error bars show the standard deviation.

**Fig. 4A Inhibition of *S. pseudintermedius* adherence to fibronectin using fibronectin fragments.** *S. pseudintermedius* was incubated with increasing concentration of fragments of fibronectin corresponding to the gelatin-binding domain ( $\circ$ ), the cell-binding domain ( $\blacktriangle$ ) and the N-terminal 29 kDa (N29) domain ( $\bullet$ ) before

being added to the wells of a microtitre plate coated with fibronectin (5 µg/ml).

Adherent bacteria were detected by staining with crystal violet. Values are expressed as a percentage of control wells lacking inhibitor peptide. Results shown are the mean values of triplicate samples.

**Fig. 4B Adherence of *S. pseudintermedius* 326 and *S. aureus* SH1000 to immobilised N29.** Plates were coated with doubling dilutions of the 29 kDa N-terminal fragment of fibronectin (N29). *S. pseudintermedius* 326 (○) and *S. aureus* SH1000 (●) were added. Results shown are the mean values of triplicate samples. Error bars show the standard deviation.

**Fig. 5. Fibronectin-affinity blotting analysis of cell-wall extracts from *S. pseudintermedius*.** Total cell-wall extracts of *S. aureus* Newman (1), *S. aureus* P1 (2), *S. pseudintermedius* 326 (3) and *S. aureus* SH1000 (4) were separated on 7.5 % acrylamide gels and electroblotted onto PVDF membranes. Membranes were probed with a solution of biotinylated fibronectin. Size markers are indicated.

**Fig. 6. Southern hybridisation of *S. pseudintermedius* genomic DNA to the SD repeat probe.** Hybridisation of *Spe*I-cleaved genomic DNA of *S. pseudintermedius* to SD repeat probe. Size markers are indicated.

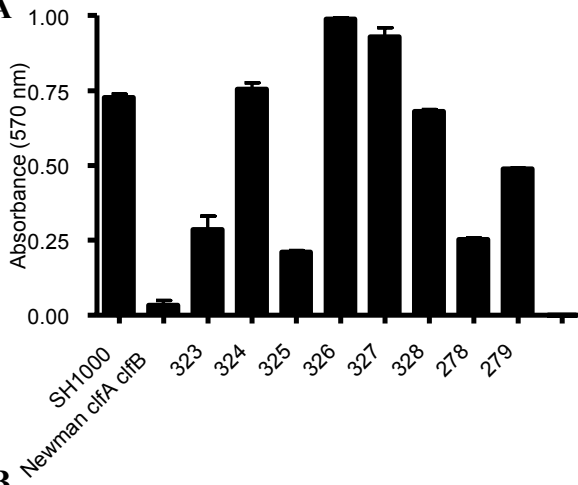
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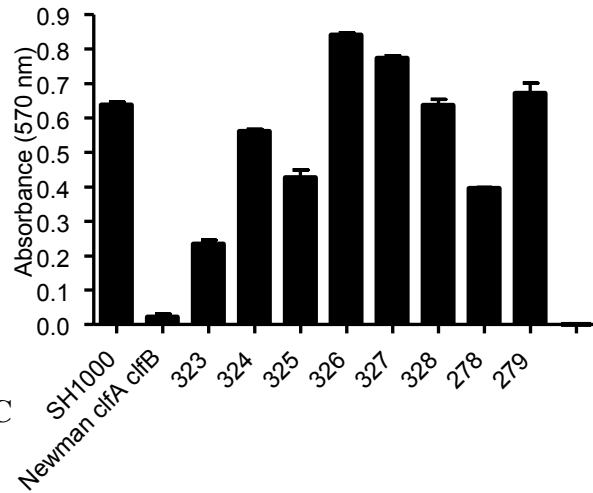
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**FIG. 1A**



**FIG. 1B**



**FIG. 1C**

