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Slime production is essential for the adherence of *Staphylococcus epidermidis* in implant-related infections

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SUMMARY

A total of 32 *Staphylococcus epidermidis* isolates from indwelling device-related infections such as endophthalmitis following intraocular lens (IOL) implantation, intravenous catheter-related sepsis and orthopaedic implant infections, were studied for slime production and adherence to artificial surfaces. Of these, 21 (65.6%) isolates were slime positive by the Congo Red agar method and 24 (75%) were adherent to artificial surfaces by the quantitative slime test. The majority (19 out of 24; 79.1%) of the adherent bacteria were slime producers. Antibody to slime raised in rabbits was able to inhibit the adherence of all 24 bacteria designated as adherent by our quantitative test. It seems that slime is indispensable for the sessile mode of attachment, leading further to the development of biofilms on the indwelling devices.

Introduction

Slime is an exopolysaccharide liberated by *Staphylococcus epidermidis*.[1] The clinical significance of slime from *S. epidermidis* in device-associated infections has been documented.[2] Slime has also been reported as a virulence factor of *S. epidermidis* in bacterial keratitis.[3,4] Clinical and laboratory evidence have supported the view that slime-producing *S. epidermidis* isolates from cases of keratitis are adherent to artificial surfaces.[3,5] The molecular basis of slime production was established by amplification of the '*ica*' locus using polymerase chain reaction assay in *S. epidermidis* isolates from central venous catheter-related sepsis.[6] Despite all of the clinical, epidemiological, laboratory and molecular data, the exact role of slime as an adhesin has not been elucidated.[2–6]

The present study was undertaken to assess whether such slime exopolysaccharide produced by *S. epidermidis* in implant-associated infections has any direct adhesion potential, which might be involved in the process of attachment on to the indwelling implants.

Methods

Subjects and case definitions

Samples were collected from 73 patients with indwelling-implant related infections. They included 39 subjects of postoperative endophthalmitis following intraocular lens (IOL) implantation, 24 subjects with intravascular catheter-related sepsis, and 10 having infections due to implanted orthopaedic devices.

Individuals with features of endophthalmitis such as increasing pain and redness, decreasing visual acuity, flare in the anterior chamber, corneal oedema, hypopyon, and poor glow within four to six weeks following IOL implantation were classified as cases of late-onset postoperative endophthalmitis (POE). All 39 subjects fulfilled the above criteria.

The 24 patients with indwelling central venous catheters/intravascular cannulas had clinical evidence of infection (catheter-related sepsis) with fever $\geq$38 °C, pulse rate of >90/min, respiration rate of >20/min and white blood cell count of >12 000/mm^3 of blood. In addition, subjects with localised infection on the exit of the truncated tract were also considered as having catheter-related infections.

Those who were having either localised inflammatory signs at the implant site or signs of sepsis, as defined above, owing to

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implantation of joint prostheses, nails, plates and bone cements were said to have orthopaedic implant-associated infections.

Sample collection and processing

Patients with POE

About 0.1 mL of vitreous fluid was collected with the help of sterile tuberculin syringe and 26 gauge needle. The bevelled tip of the needle was closed with a sterile rubber bung and was transported to the laboratory immediately. The vitreous fluid was stained and cultured according to the standard procedures.

Patients with intravascular implants

The tips of intravascular catheters/cannulas and/or central venous catheters were collected aseptically, in sterile test tubes, and were immediately inoculated on to blood agar plates according to the rolling technique of Maki *et al.* and subsequently on to trypticase soy broth (TSB).[7] The agar plates and TSB tubes were incubated at 37 °C. For blood culture, the sample of blood was inoculated directly on to the broth.

Patients with orthopaedic implants

Aspirate was collected with sterile syringe and needle after properly disinfecting the surrounding skin. Pus or discharge was collected by rubbing the bed of the ulcer with a sterile cotton-tipped swab. If the material was insufficient, then the wound was squeezed and the exuded purulent material was collected. Intra-operative pus, if obtained, was directly inoculated on to TSB. Blood was also collected for culture if there was indication of sepsis. Additionally, the pus, aspirate, discharge were subjected to Gram staining.

The material inoculated into TSB was incubated at 37 °C. Growth from TSB was subcultured on to blood agar, MacConkey agar and chocolate agar plates, which were incubated at 37 °C.

Identification

After overnight incubation, those colonies showing Gram-positive cocci on smear examination were processed further. The organisms were identified and speciated according to the previously described method and were stored at −20 °C as nutrient agar stab cultures until further testing.[4]

Slime test

Test for slime production was carried out by the Congo Red agar (CRA) plate method described earlier.[8]

Quantitative slime test for adherence

Adherence of each isolate to smooth surfaces was determined quantitatively by a method earlier standardised in our laboratory.[5]

Briefly, our procedure was as follows. Overnight cultures of bacteria in trypticase soy broth (TSB) were diluted 1 in 100 in fresh TSB, and 1 mL volume of each isolate was put into separate quartz cuvettes. After overnight incubation at 37 °C, the cuvettes were washed four times with phosphate-buffered saline (PBS), fixed with Bouin's fluid, and stained with crystal violet. Excess stain was removed by decanting the tubes and then rinsing them gently with tap water. The optical density (OD) of the stained adherent bacterial film was read with a spectrocolorimeter (Spectrocolorimeter 103, Systronics, Baroda, India) at 570 nm. Organisms were considered non-adherent if the OD was below the cut-off recorded for that batch of experiments. This cut-off was calculated as 3× SD above the mean OD of 10 blank cuvettes stained by the above-described procedure in that particular batch of tests.

Extraction of slime

Crude slime was extracted by a procedure previously described.[9] Briefly, 50 mL of mid-log phase of bacterial suspension were inoculated in 1 L of TSB and incubated for 24 h at 37 °C, in humidified chambers. Extracellular material was removed from the cells by gentle shaking with glass beads in 0.15 M NaCl. The extracts were precipitated with a mixture of ethanol, sodium acetate and acetic acid in final concentrations of 80%, 0.26 M and 0.05 M respectively. The precipitate was dissolved in distilled water, the insoluble material was removed by centrifugation at 27 000 *g* for 30 min, and the supernatant was dialysed three times against 100 volumes of distilled water. The above procedure was repeated twice. The dialysates were centrifuged at 105 000 g for 3 h and the supernatants were freeze-dried and stored at −70 °C. All of the above steps were performed at 4 °C in the presence of 6-aminohexanoic acid, EDTA and phenylmethane sulphonylfluoride at final concentrations of 50, 10 and 20 mM respectively.

Antibodies to slime

Rabbit antiserum to slime was prepared by immunisation of the animals by a protocol earlier adapted by Karamanos *et al.* with some modifications.[10] Filter-sterilised antigen, i.e. crude slime, was emulsified with equal volumes of Freund's complete adjuvant for the first injection subcutaneously and incomplete Freund's adjuvant for the subsequent challenges one week later intravenously thrice weekly for three weeks. Animals were bled five days after final injection and antibody titres were measured by enzyme-linked immunosorbent assay.

Adherence inhibition test

Quartz cuvettes were coated with 1:20 dilution of the immune sera at 4 °C overnight, by addition of 1 mL of diluted serum to each cuvette. Subsequent steps followed were the same as those described for the quantitative adherence test, the only exception being that bacterial suspension was added to antibody-coated cuvettes.[5] The OD values recorded after such experiments (adherence inhibition test) were plotted for comparison with those recorded earlier (adherence test).

Results

Culture positivity in samples collected from patients with various implants

Overall culture positivity was found in 43 (58.9%) samples, out of which *S. epidermidis* grew in 32 (28 as pure growth and four as mixed growth) (Table I). Other organisms recovered were: five isolates of diphtheroids from the vitreous fluid; two *Pseudomonas aeruginosa*, one *Candida albicans* and two viridans streptococci, all from intravascular catheters/cannulas; and a single isolate of *Staphylococcus aureus* from a pus sample in a patient with orthopaedic implant. All blood cultures were sterile. However, other organisms isolated as mixed growth along with *S. epidermidis* were *Escherichia coli* in two specimens (one from catheter and the other from pus), *S. aureus* in one and *P. aeruginosa* and *E. coli* in one specimen.