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S. Aureus Subcutaneous Infection with Aggregated Bacteria

Sven Åke Hedström

Associate Professor Emeritus, Specialist in Infectious Diseases, Dept of Infect Clinic, Lund University Hospital, Sweden

***Corresponding author**
Sven Åke Hedström, MD, PhD, Associate professor emeritus, specialist in Infectious Diseases, Dept of Infect Clinic, Lund University Hospital, Sweden. E-mail: svenakehedstrom@gmail.com

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Introduction

Staphylocooccal infections are common and can engage several tissues and organs of the body. Some decades ago we had antibiotics which were effective and commonly used against S. aureus infections, i e penicillin V, G and isoxazolylpenicillins, but resistance occurred with time. Also with full sensitivity, some staphylococcal infections were a challenge to treat, such as heart valvular infection, skeletal infection etc. Mostly, skin and soft tissue infections responded well to antibiotic treatment.

In the following a case of subcutaneous S. aureus infection is presented which for an unexpectedly long time was unknown as a staphylococcal infection, although treated as such there was no success in healing. Previous experimental studies related to the bacteriological findings from the patient are also presented.

Case report

A 64 years old man IA underwent in 1981 at the University Hospital in Lund, Sweden, a partial resection of the right lung due to lung cancer. An uncomplicated healing of the wound started but after a couple of days the patient was admitted back to the Thoracic Surgery Clinic with a lateral fistula in the surgical area (Figure 1). Cultures showed repeatedly only growth of Staphylococcus albus. Isoxalylpenicillin therapy in a normal dosage for soft tissue infection was started at the same time as suction drainage through the fistula without diminishing of the purulent discharge. The patient was transferred to the Clinic of Infectious Diseases. Smears for microscopical diagnosis were taken. Gram staining and methylenblue staining were performed, the results are commented in the coming section. Ordinary cultures were repeated, only S. albus were found. Flucloxapenicillin in a higher dosage was given. No improvement was noted. New fistular material was aspirated for staining and also for culture in rotatory bottles with trypticase soy broth at 37 degrees Celsius. This method revealed S. aureus.

Figure 1: Postoperative subcutaneous infection

An addition of local treatment through the fistula was started. A mixture of dextrane containing 5% cloxacillin for parenteral use was administered morning and evening via a catheter inserted through the fistula to the subcutaneous cavity [1]. The amount of secreted pus diminished rapidly during the following days, the fistula closed and the patient IA could be discharged healed to his home.

Microscopy of the smears

In a light microscope the methyleneblue staining showed an even mat of leukocytes in which some remarkable structures were detected (Figure 2a, Figure 2b). These were intensively stained in the central part which was surrounded by apparently granulocytes in a rosette form (Figure 3). In another structure a higher solution of the central part seemed to consist of aggregated bacteria, astonishing as bacteria in chain structures radiating out in the periphery (Figure 4).

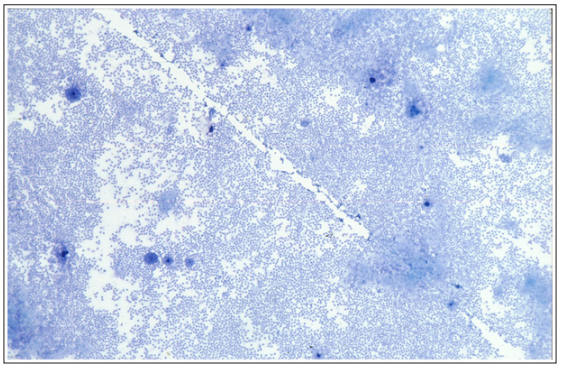

Figure 2a

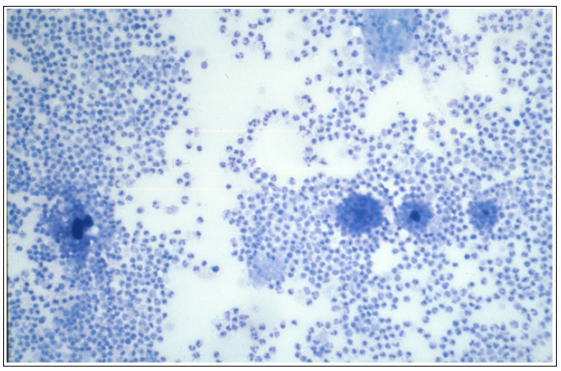

Figure 2b

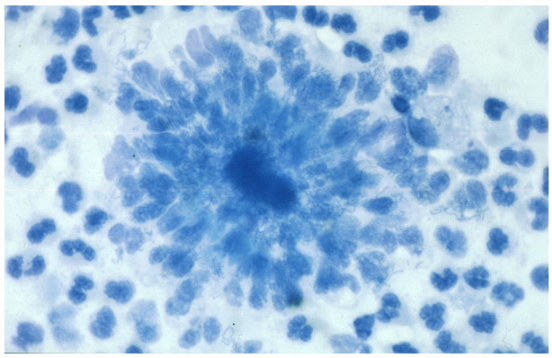

Figure 3

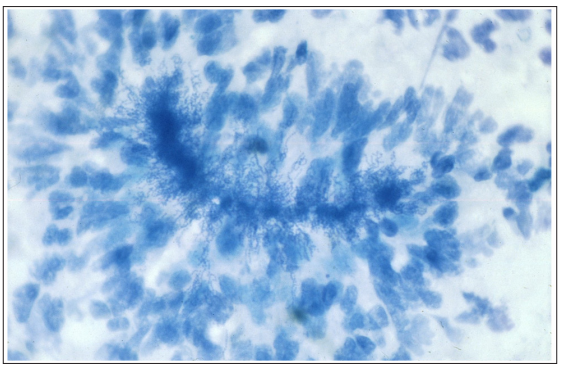

Figure 4

Previous research

Cultivation of S. aureus in lipid emulsion

With the aim to study the interaction between S. aureus and triglycerides considering the lipase activity, changes in bacterial morphology were found. Various strains were cultivated in trypticase soy broth containing 1% triglyceride of oleic acid in shake bottles at 37 degrees Celsius for 24 hours and a small amount was taken for smears stained with methylenblue or defatted with ethanol and after drying the smear stained with picric acid (Figure 5). The borders of the former fatty vacuoles seemed to be lined with bacteria in chains.

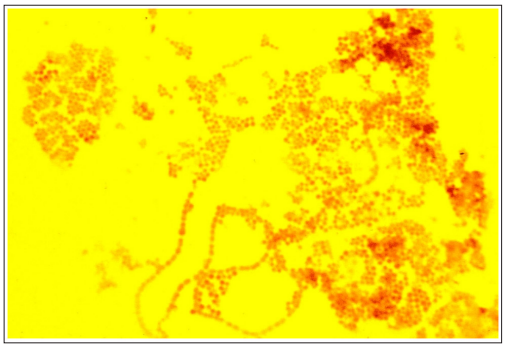

Figure 5

Electron microscopy studies

Material from growth of bacteria in 1% intralipid trypticase soy broth emulsion were studied in Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM). Control studies used S aureus in broth without lipid. The aim was to look for morphological changes of the staphylococci grown in the presence of triglyceride. Beside ordinary rounded shape of the Staphylococcus elongated structures were revealed in SEM, in some sites aggregated (Figure 6a, Figure 6b). In TEM some structures without the staphylococcal configuration were noted (Figure 7), the bacteria were somewhat elongated. In broth without triglycerides no structures like the described ones could be seen in ordinary microscope or at SEM.

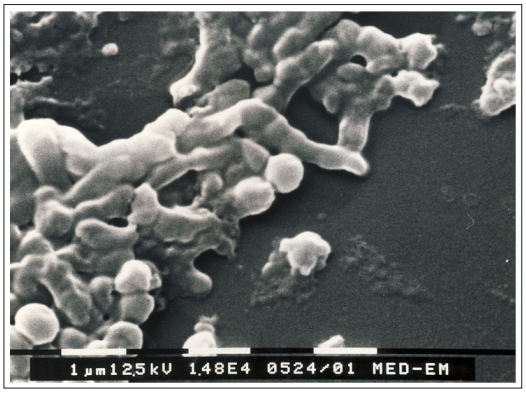

Figure 6

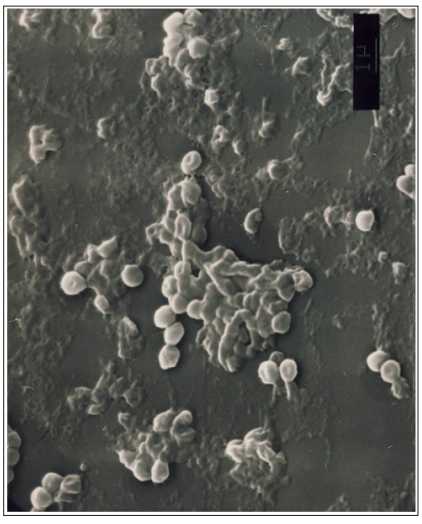

Figure 6b

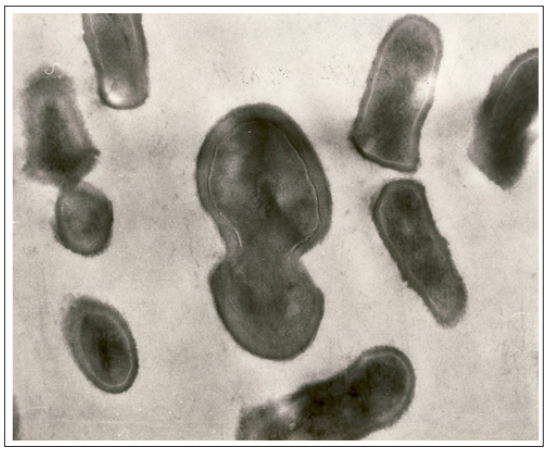

Figure 7

Staphylococcus aureus lipase activity

Lipase is one of the common enzymes produced by S. aureus. There are differences in the amount of lipase production. Strains from deep or subcutaneous infections i.e. septicemia, pyomyositis, osteomyelitis had a greater production and significantly higher lipase activity than those isolated from superficial infections like impetigo and carriers in nasal mucosa [2].

Several S. aureus strains in radiolabelled (oleic acid) trioleinphospholipid broth emulsion showed accumulation of fatty acids in the lipid broth in various amounts due to variations in lipolytic activity by the strains rather than incorporation in the staphylococcal strains [3]. An incorporation of radiolabelled oleic acids in the cell structure was shown in 4 studied strains. The lack of production of 14C-labelled carbon dioxide by the strains indicated that the oleic acid was not used as an energy source[3].

Discussion

In the seventies the author investigated the interaction between Staphylococcus aureus and triglycerides in vitro considering the lipolytic activity and possible changes of staphylococcal morphology.and changes such as in Figure 5 were found. The findings were discussed with microbiologists who interpreted them as artefacts. Therefore the findings were not published. In 1981 the author had a patient which is presented here. His infection was resistant to initial antibiotic therapy and first with treatment with high local administration of an antistaphylococcal drug the infection healed. Astonishing, similar structures as in the in vitro investigations were found in smears of purulent material from the patients subcutaneous infection. A theoretical explanation could be that the subcutaneous fat tissue was lysed by the bacterial lipase to fatty acids behaving as a possible factor for interaction with staphylococcal growth. The case report has been presented only as a non-published lecture in Sweden. A similar case was mentioned at that time, a woman with chronic osteomyelitis presenting as a fistula down to the femur. Also in this case a few small aggregations of staphylococci surrounded by granulocytes in a rosette form were detected in the stained smears. The interaction between S. aureus and fatty tissue is worth a thorough investigation.

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