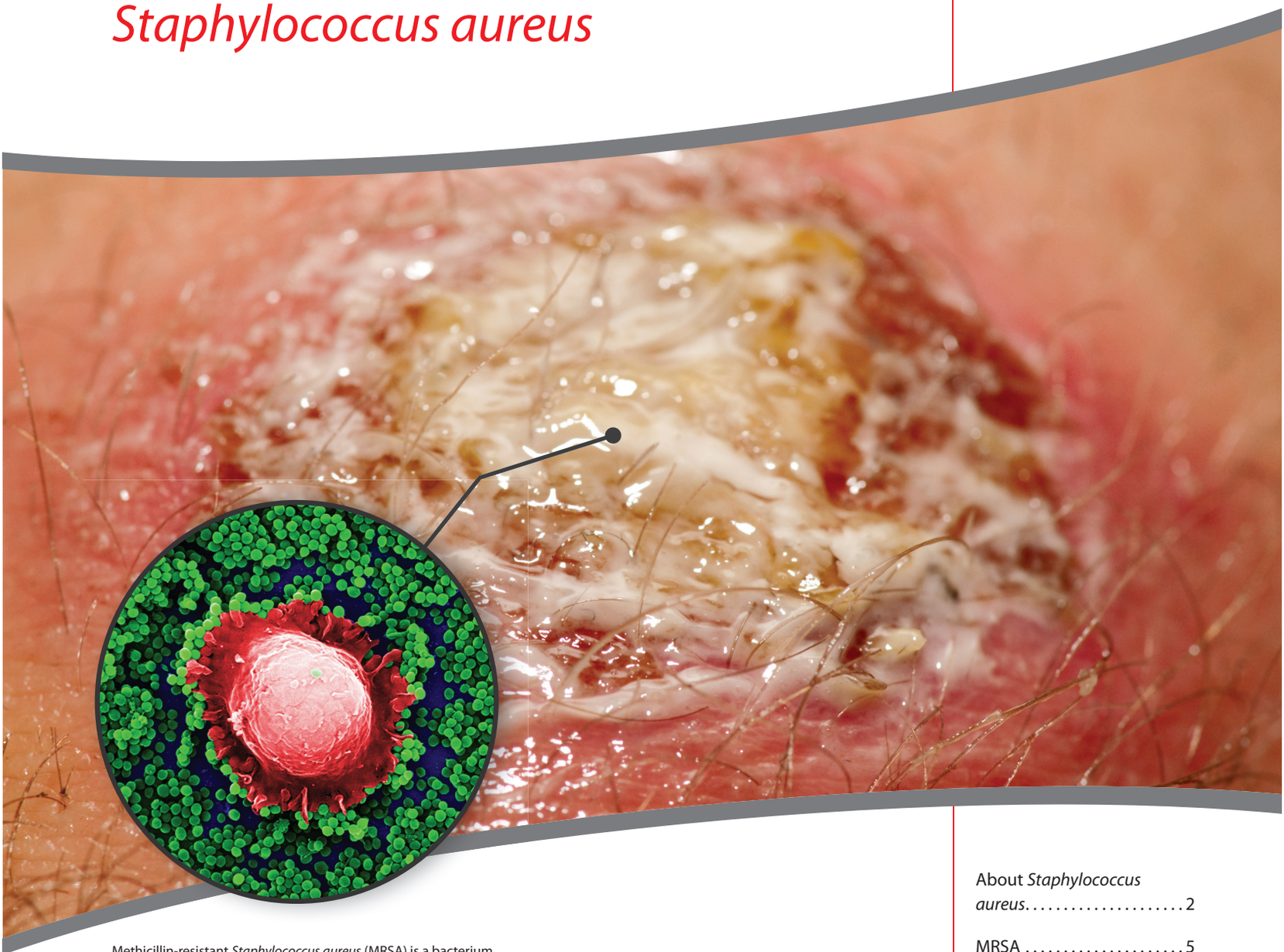
Microbiology Focus

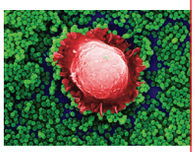
Volume 6.1, 2014

Fluka® Analytical

The Role of *Staphylococcus aureus*



Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterium responsible for several difficult-to-treat infections in humans. The ability of MRSA to establish biofilms has been linked to the persistence of chronic infections. The colored microscopic image shows a macrophage grazing in a MRSA biofilm (magnification ×10.000).



About *Staphylococcus aureus*

Jvo Siegrist, Product Manager Microbiology — *ivo.siegrist@sial.com*

Staphylococcus aureus is nearly everywhere in nature and 25 to 30% of the population has it on the skin, hair and/or in the upper respiratory tract.

Staphylococcus aureus is found in both animals and humans. It can cause food poisoning when a food handler contaminates food and then the food is not properly refrigerated. Other contamination sources include the equipment and surfaces in hospitals or in the food industry. *S. aureus* is still one of the five most common causes of nosocomial infections, often causing postsurgical wound infections. In recent years, *S. aureus* is becoming famous because of its resistance to antibiotics, mainly methicillin, and in most cases to all related antibiotics (β-lactam antibiotics). Therefore, the resistant strains are called methicillin-resistant *Staphylococcus aureus* (MRSA) or also oxacillin-resistant *Staphylococcus aureus* (ORSA). Nowhere has this increase in multi-resistant strains been of greater concern than with the Gram-positive bacteria like pneumococci, enterococci and staphylococci.[1]

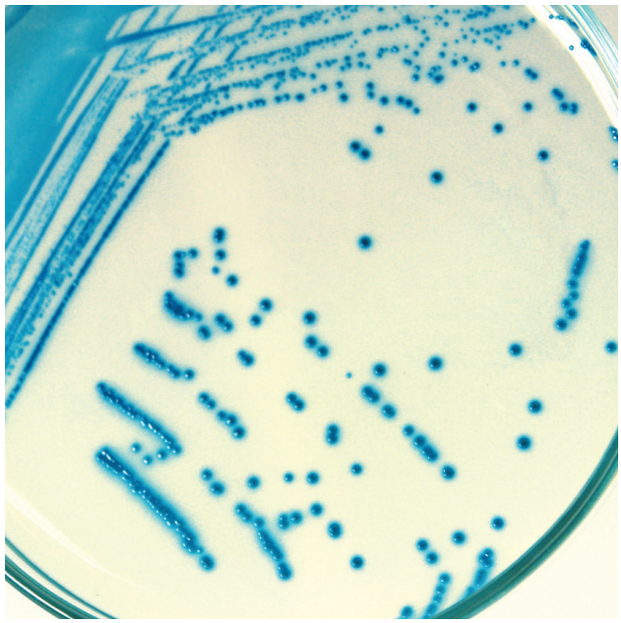


Figure 2: MRSA on HiCrome™ MeReSa Agar (Cat. No. 90923)

Test Strains	Origin	Strain #	CFU	Cat. No.
S. aureus susp. Aureus	ATCC	6538	50	RQC13002-10EA
S. aureus susp. Aureus	ATCC	6538	200	RQC13005-10EA
S. aureus susp. Aureus	ATCC	6538	1'000	RQC13007-10EA

Table 1: Vitroids™ the certified reference microorganisms in different colony forming units (CFU) levels.

Did you know…

Staphylococcus aureus builds biofilms to protect itself?

In a biofilm (on any surface), the bacteria organize and build themselves protective substances like polysaccharides to elude attacks by antimicrobial agents such as antibiotics.

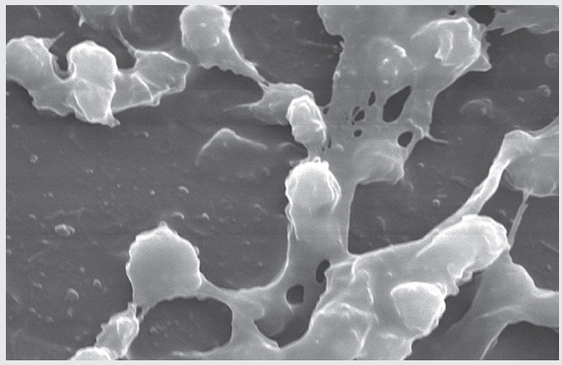
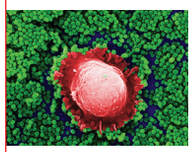


Figure 1: *S. aureus* bacteria found on the luminal surface of an indwelling catheter. The sticky-looking substance woven between the round cocci bacteria is composed of polysaccharides, and is a sign of a biofilm. (Source: CDC; Rodney M. Donlan, Janice Carr, 2005)

S. aureus has a typical circular shape and is Gram-positive. They do not move actively and cannot build spores. The cells usually build a grape cluster berry-like group, which gave them the name *Staphylococcus* from the Greek words "staphylé," meaning a bunch of grapes, and "coccus," which means round-shaped. "Aureus" is Latin and means "golden", because most colonies have a characteristic orange-yellow coloring on the traditionally used agar plates, and *S. aureus* is responsible for the golden-yellow pus indicating infection. They are relatives of lactic acid bacteria, and the fermentation of these facultative anaerobic pathogens also ends as lactic acid. Therefore, it is no wonder that they can live in the same nutrients as lactic acid bacteria. They can grow in the temperature range of 15–45 °C and in a medium with up to 15% sodium chloride. At room temperature, *S. aureus* multiply quickly but they are killed by cooking food properly. The main issue resulting from food poisoning caused by *S. aureus* is the production of several relatively heat stable exotoxins. The exotoxins can be categorized into three groups: the superantigens, the exfoliative toxins along with a group of other toxins which act on the cell membranes and the bicomponent toxins.[2]



Handmade foods without additional heat processing:
Salads made with ham, egg, tuna, chicken, potato or macaroni
Bakery products containing cream
Sandwiches
Other sources:
milk and dairy products
meat, poultry, eggs and related products

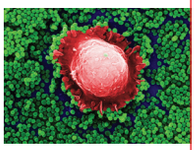
Table 2: Common high-risk food sources

A wide range of media employing selectivity and biochemical differentiation systems may be used for the detection and identification of *S. aureus* (**Table 3**). Classical media like the ISO recommended Baird Parker RPF Agar or chromogenic media such as HiCrome Aureus Agar (Cat. No. 05662), HiCrome MeReSa Agar (Cat. No. 90923, see **Figure 2**) and Phenolphthalein Phosphate Agar (Cat. No. 68879) are currently available and used for detection and enumeration. Lithium chloride, sodium azide, tellurite and sulphamezatine may serve as selective agents in selective media. More details regarding the classical cultural method can be found under the ISO methods ISO 6888-1:1999 + A1:2003, ISO 6888-1:1999 + A1:2003 and ISO 6888-3:2003. Additionally, there are diverse biochemical characteristics for the confirmation and identification of *S. aureus*. A flowchart of one of the most common and easiest identification pathways appears in **Figure 3**. As usual, the recommended starting point is to check the shape of the bacteria under the microscope and the Gram coloration. The second step is a catalase test, followed by any of a diverse range of other individual tests or media that are available (**Tables 3, 4 and 5**). An immunological test such as the Staphylo Monotec test kit Plus (Cat. No. 50448), a spot agglutination test, is also often used for confirmation. This kit has been evaluated for clinical specimens and food material. With this kit, coagulase, protein A and capsular Polysaccharide (serotype 5) on *S. aureus* can be detected in one step, which gives a highly reliable result. Compared to the other classical spot test, this kit has an increased sensitivity and specificity, which mainly results in detecting more methicillin-resistant *S. aureus* (MRSA).

Identification Media	Cat. No.	Testing Features
Azide Blood Agar (Base)	70132	Detection of β-hemolysis
Baird Parker Agar*	11705	Detection of lipolytic and proteolytic activity, ability to reduce tellurite to metallic tellurium (ISO 6888-1:1999, ISO 6888-3:2003); with RPF Supplement the coagulase activity and the ability to reduce tellurite is detected (ISO 6888-2:1999, ISO 6888-3:2003)
Baird Parker Agar Base (RPF) Supplements: Egg-Yolk Tellurite Emulsion (Cat. No. 75208) RPF Supplement (Cat. No. 05939)	79893	
Blood Agar Supplement: defibrinated blood	70133	Detection of β-hemolysis
Blood Agar No. 2 Supplement: defibrinated blood	B1676	Detection of β-hemolysis
Brain Heart Infusion Broth	53286	For confirmation with rabbit plasma (ISO 6888-3:2003)
Bromo Thymol Blue (B.T.B.) Lactose Agar	B3676	Differentiated by their ability to grow at a high pH and in the presence of bromo thymol blue (golden yellow colonies)
CLED Agar	55420	Detection of lactose fermentation
Deoxyribonuclease Test Agar	30787 70136	Detection of deoxyribonuclease activity
DNase Test Agar with Toluidine Blue	D2560	Detection of deoxyribonuclease activity
Giolitti Cantoni Broth, Modified (ISO) Supplement: Potassium Tellurite Solution (Cat. No. 17774)	69527	Selective enrichment acc. ISO 6888-3:2003
HiCrome™ Aureus Agar Base* Supplement: Egg-Yolk Tellurite Emulsion (Cat. No. 75208)	05662	Testing for ability to reduce tellurite to metallic tellurium and detection of lipase and protease by chromogenic substrate; brown-black colonies
HiCrome™ MeReSa Agar Base* Supplement: MRSA Selective Supplement (Cat. No. 51387)	90923	Detection by chromogenic substrate mixture specifically cleaved by *S. aureus*; selective to MRSA (bluish green colonies)
China Blue Lactose Agar*	22520	Detection of lactose fermentation
Mannitol Salt Phenol Red Agar	63567	Detection of mannitol fermentation in high sodium chloride concentration
Nutrient Gelatin	70151	Detection of gelatin-liquefying (proteolytic enzymes)
Phenolphthalein Phosphate Agar	68879	Phosphatase detection; pink-red colonies
Spirit Blue Agar Supplement: Lipase Substrate (see data sheet)	S4306	Detection and enumeration of lipolytic activity
Staphylococcus Agar*	70193	Detection of salt tolerance, pigmentation, D-mannitol utilization and gelatin liquefaction
Tributyrin Agar Supplement: Neutral Tributyrin (Cat. No. 91010)	91015	Detection and enumeration of lipolytic activity
Vogel-Johnson Agar Supplement: Potassium Tellurite 1% (Cat. No. 17774)	70195	Checking for ability to reduce tellurite to tellurium and ability to ferment mannitol

Table 3: Media for detection and identification of *S. aureus*

*Not available in U.S.A.



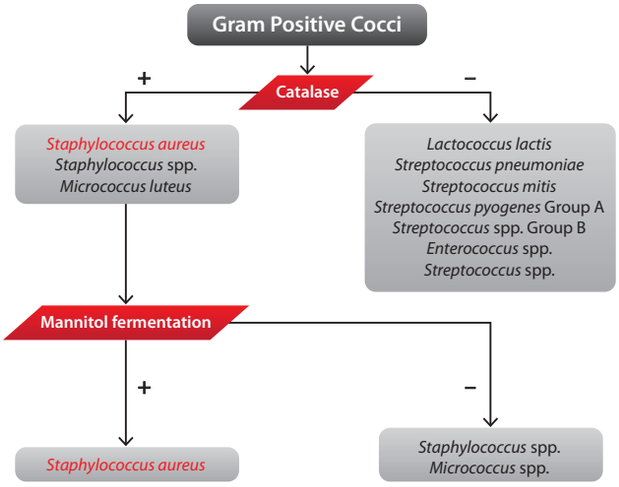


Figure 3: ID flowchart for *S. aureus* (Bergey's Manual)

Characteristic Properties	Result	Tests
Higher peptidoglycan and lower lipid content in cell wall	+	Gram coloration
Catalase	+	H_2O_2 test
Facultative anaerobe	+	TSA Deeps
Oxidase	–	
Coagulase	+ (97%)	Clotting with fibrinogen
Protein A	+ (95%)	Immunological
Capsular Polysaccharide (serotype 5)	+	Immunological
Lecithinase	+	Egg yolk-lecithinase reaction (e.g. Baird Parker Agar)
Reduction of tellurite	+	Reduction to tellurium (e.g. Baird Parker Agar)
DNase	+	DNase test
Mannitol fermentation	+	
β-Hemolysis	+ (mostly)	Blood Agar
Phosphatase	+	Phenolphthalein Phosphate Agar
Proteolytic enzymes	+	Nutrient Gelatin

Table 4: Biochemical characteristics of *S.aureus*

Kits and Tests	Cat. No.
Catalase Test (Hydrogen peroxide 3%)	88597
Coagulase Test (Slide)	75832
Coagulase Test (Tubes)	74226
Gram Staining Kit	77730
Mannitol Disks	94438
Oxidase Reagent acc. Gaby-Hadley A + Oxidase Reagent acc. Gaby-Hadley B	07345 + 07817
Oxidase Reagent acc. Gordon-McLeod	18502
Oxidase Strips	40560
Oxidase Test	70439
Staphylo Monotec test kit Plus	50448

Table 5: Test for detection and identification of *S. aureus*

Figure 4: Staphylo Monotec test kit Plus (Cat. No. 50448) with a positive agglutination reaction

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Host-Pathogen Interactions in Methicillin-Resistant *S. aureus* (MRSA)

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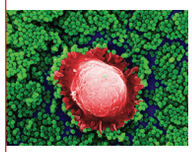


Staphylococcus aureus is a major human pathogen. Methicillin-resistant *Staphylococcus aureus* (MRSA) is an *S. aureus* that became resistant to many antibiotics introduced into clinical use in the past half century. Three main mechanisms contribute to resistance in *Staphylococcus aureus*. These are the production of a supplemental penicillin-binding protein (PBP) that is encoded by a chromosomal *mecA* gene; the hyper ß-lactamase production, and the production of modified PBPs, which lowers the organisms affinity for ß-lactam antibiotics (**Figure 1**). The continuous emergence and spread of MRSA strains mean treatment options are limited. Compared with methicillin-susceptible *S. aureus* (MSSA), MRSA is known to cause higher morbidity and mortality and, therefore, an accurate susceptibility testing of *S. aureus* isolates and screening of patients for colonization with MRSA are important tools to limit the spread of this organism.

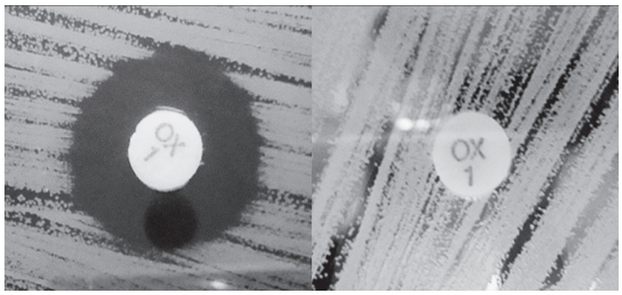


Figure 1: Sensitivity and resistance to Oxacillin in a *S. aureus* reference strain (left) and a clinical isolate (right) respectively.

The bacteria have been classified into two categories based on where infection is first acquired-Community-Associated MRSA and Hospital-Acquired MRSA. Outbreaks of community-associated MRSA have involved bacterial strains with specific microbiologic and genetic differences from hospital-acquired MRSA strains, and these differences suggest that community strains might spread more easily from person to person. Hospital-acquired MRSA primarily affects people in healthcare settings, such as those who have had surgery or implanted medical devices. This source of MRSA is typically problematic for people with weakened immune systems and for the elderly.

Vancomycin has been used for years to treat MRSA infections, but there is increasing evidence that this drug only provides suboptimal treatment of bacteremia and other serious infections caused by MRSA, with MICs of vancomycin near the breakpoint for susceptibility to this compound. Linezolid, another compound used against MRSA, may be of importance when treating serious infections by these bacteria, however, its activity against MRSA is only bacteriostatic. In these circumstances, the clinical potential of new drugs for treating MRSA infections is of major interest.[1] Moreover, despite the fact that *S. aureus* is classically considered extracellular, certain features of staphylococcal disease suggest that this organism has the capacity to function as an intracellular pathogen.[2] Adherence of *S. aureus* to host structures is a prerequisite for asymptomatic colonization and overt disease, and, in common with several other bacterial pathogens, *S. aureus* invades a variety of non-professional phagocytic cells *in vitro*. Adherence has been shown to be related to the expression of bacterial surface fibronectin-binding proteins and host cell integrins.[3,4] Epithelial cells play an important role as the interface between the host mucosal surfaces and the surrounding environment and are the initial site of colonization for bacterial pathogens. MRSA could gain entry in either epithelial or other types of non-phagocytic cells, thereby avoiding the host hostile environment and disseminating more easily like other intracellular pathogens do.[5] However, it is not clear whether intracellular persistence of MRSA also occurs in human infections. This is a subject of intense debate which is also difficult to assess experimentally. In addition to this problem, antibiotic therapy for MRSA infections is becoming more difficult in hospitals because of strong biofilm-forming properties. Staphylococci, especially MRSA strains, are the most frequent cause of biofilm-associated infections, which are a significant cause of morbidity and death and associated with indwelling medical devices.[6] They are also associated with a number of diseases including endocarditis, cystic fibrosis and nosocomial diseases related to catheters, prosthetic heart valves and orthopedic devices.

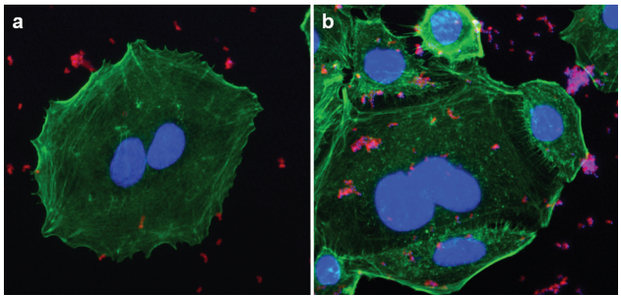
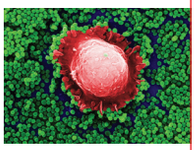


Figure 2: Adherence of MRSA to human epithelial cells. Cells were infected for 30 mins with a low-adherent (a) or a high-adherent (b) clinical MRSA strain. Bacteria were detected with anti-*S. aureus* rabbit antibody (shown in red-purple). Fluorescently labelled phalloidin (Atto-phalloidin 488, Sigma), which binds polymerized f-actin, was used to identify actin filaments and fibers. DAPI-stained nuclei are shown in blue. Micrographs were originally captured at ×400 magnification.

Biofilm formation proceeds in several phases, from primary attachment and proliferation to microcolony formation. Proliferation proceeds through the production of biofilm matrices that contribute to bacterial accumulation in multiple layers where cells are embedded in extracellular matrices composed of proteins, sugars, and extracellular DNA.[7,8] If progress is going to be made in treatment and restoration of patient health, it is also necessary to gain a better understanding of what occurs between the immune system's cells and MRSA biofilms. As our laboratory is devoted to the study of host-pathogen interactions in clinically relevant bacteria, the interactions of human epithelial cells and mouse macrophages with MRSA and MRSA biofilms were analyzed by immunofluorescence and scanning electron microscopy.



Among several MRSA clinical strains, inter-strains variability exist with respect to their degree of adhesion to epithelial cells *in vitro*. An example is shown in **Figure 2**. Epithelial cells were colonized in some cases by clumped bacteria, which showed a localized pattern of adherence, or by individual bacterial cells. SEM microphotographs showed that several strains were able to penetrate the epithelial cells. The most frequent morphology showed a well defined zipper-like mechanism, in which the host cell membrane was zippered around the bacterium while entering the host cell (**Figure 3**). Using macrophages as host cells, we observe that macrophage phagocytosis attempts to engulf live MRSA strains were successful before 30 min of infection *in vitro* (**Figure 4a**). Also, macrophages engulf biofilm cells very easily (**Figure 4b**). We believe that development of efficacious therapeutic interventions against MRSA will need to consider host-pathogen interactions to enhance host immunity, to circumvent bacterial evasion strategies and to fight biofilm strength.

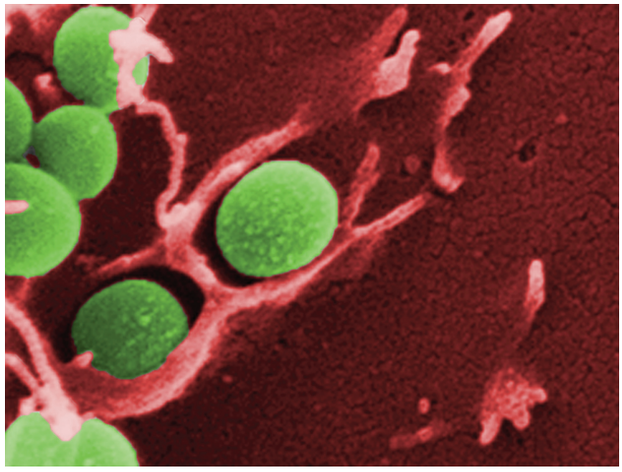


Figure 3: Internalization of a *S. aureus* strain in human epithelial cells. Cells were infected, fixed and processed for SEM. The pseudocolored microphotograph shows two bacteria penetrating a cell after the generation of membrane invaginations at the level of the bacterial-cell contact area. Micrograph was originally captured at ×15.000 magnification.

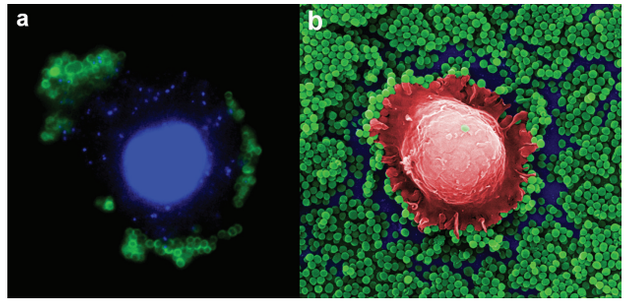


Figure 4: a) Immunofluorescence microphotography of a macrophage filled with intracellular MRSA (blue dots). Extracellular bacteria were detected with anti-*S. aureus* rabbit antibody (shown in green). Micrograph was originally captured at ×400 magnification. b) This picture shows a pseudocolored scanning electron microphotography of a macrophage grazing in a MRSA biofilm. Micrograph was originally captured at ×10.000 magnification.

References

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Winners of the Photo Competition

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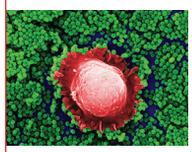
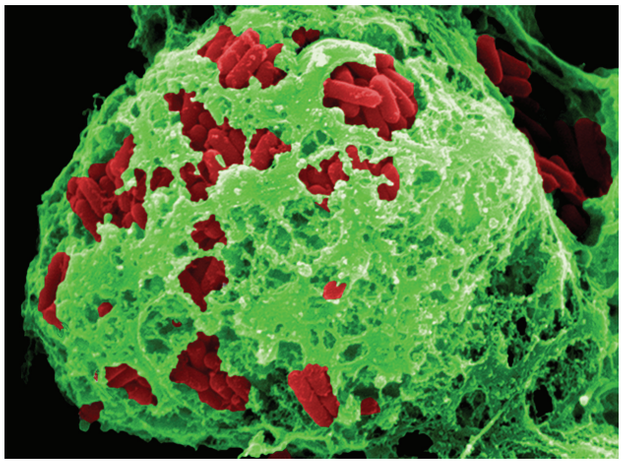
Who says that microbiology is boring? We received lots of interesting and intriguing images including ones in which bacteria blast a phagocytic cell, macrophages graze in a biofilm of *Staphylococcus aureus*, and a lot more – sounds like interesting stories. On the front cover of this issue of Microbiology Focus is the photo of the second place winner from the 2013 Sigma-Aldrich Microbiology Photo Competition. It captures a macrophage absorbing *Staphylococcus aureus*, which is the primary microorganism discussed in this issue. This is the first time that we had the same individual win both the first and second places. His name is Jose Ramos Vivas, and he is from the Instituto de Formación e Investigación Marqués de Valdecilla-IFMAV in Spain. He also wrote the article about MRSA in this Microbiology Focus. Since 2011, he has always been included among the finalists, but never won first place. This finally changed in 2013 when he surprised us with wonderful, colored scanning electron microscope images – splendidly fluorescent images with funny titles and highly interesting descriptions. His winning image will soon be featured on the cover of a future Microbiology Focus with the main topic *Listeria*.

In 2013, we received lots of fascinating images from around the world. The photographic entries winning Best of Show are featured on page 7. The complete list can be seen on our website **sigma-aldrich.com/microbiology**. The aim of the competition was to encourage microbiologists to promote their work, with the condition that entries should illustrate any microorganisms (living or dead) or a microbiologist in action at work. Sigma-Aldrich would like to thank all who entered our competition and also our independent jury members Dr. Lars Fieseler (Zurich University of Applied Sciences) and Prof. Dr. Mohammad Manafi (Medical University of Vienna).



And here are the 5 finalists:

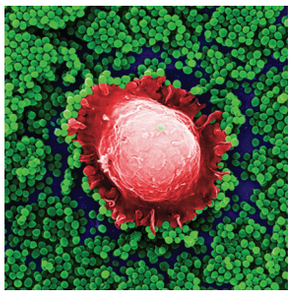
First Place: Super size me



The Scanning Electron Micrograph shows a dead, blown-up phagocytic cell that has lost large membrane areas due to a voracious appetite or to a massive intracellular proliferation of *L. monocytogenes*. Intracellular bacteria and cytoplasmic components can be observed. Original Magnification: ×20.000.

Winner of a tablet PC: José Ramos Vivas (Hospital Universitario Marqués de Valdecilla & Instituto de Formación e Investigación Marqués de Valdecilla)

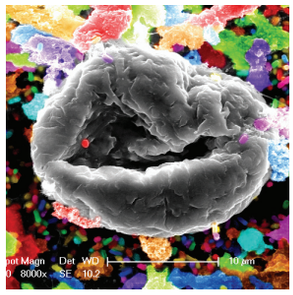
Second Place: Grazing in a *Staphylococcus aureus* biofilm



Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterium responsible for several difficult-to-treat infections in humans. The ability of MRSA to establish biofilms has been linked to the persistence of chronic infections. Biofilms can be defined as sessile communities of surface-attached cells encased in an extracellular matrix, and treatment of bacteria in this mode of growth is challenging due to the resistance of biofilm structures to both antimicrobials and host defenses including neutrophils, macrophages and dendritic cells. Our laboratory is devoted to the study of host-pathogen interaction in this and other species. This picture shows pseudocolored Scanning Electron Microphotography of a macrophage grazing in a MRSA biofilm. Original magnification ×10.000.

Winner of a MP3 player: José Ramos Vivas (Hospital Universitario Marqués de Valdecilla & Instituto de Formación e Investigación Marqués de Valdecilla)

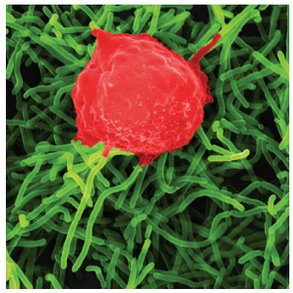
Third Place: Jaws in Candyland



A single Colpoda sp. ciliated protozoan cell. Background consists of many extracellular Listeria monocytogenes cells and co-culture debris. Instrument used: Philips XL30 Field Emission Scanning Electron Microscope at Adelaide Microscopy. Color added through Adobe Photoshop CS5. Magnification: ×8,000.

Winner of an USB flash drive: Rethish Raghu (University of Adelaide, Australia)

Fourth Place: Eating *Erysipelothrix rhusiopathiae*

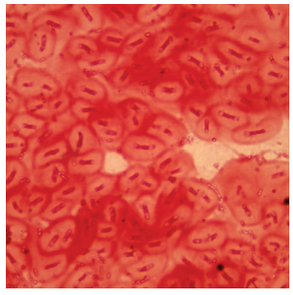


Erysipelothrix rhusiopathiae, a Gram-positive bacillus, is found widely in nature or as a commensal pathogen, and is a facultative intracellular pathogen that causes a variety of diseases in many species of birds and mammals, including humans. In pigs, *E. rhusiopathiae* can cause swine erysipelas, which may occur as acute septicemia or chronic endocarditis and polyarthritis.

This picture shows pseudocolored Scanning Electron Microphotography of a macrophage grazing *Erysipelothrix rhusiopathiae* bacteria. Original magnification ×15.000.

Winner of a laser pointer: José Ramos Vivas (Hospital Universitario Marqués de Valdecilla & Instituto de Formación e Investigación Marqués de Valdecilla)

Fifth Place: High EPS producing strain from petroleum wastes



This species was isolated from petroleum sites, unusable in diesel desulphurization. It was cultivated in Pseudomonas Agar base at pH 7.0 in presence 1%v/v mineral oil as additive. A high EPS producing species was isolated after 6 subsequent sub-cultures and designated as ABHI-PS-1.

Winner of a laser pointer: Abhilash Pillai (National Metallurgical Laboratory, India)

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