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Antibacterial effects of home-made resin salve from Norway spruce (*Picea abies*)

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Resin salve made from Norway spruce (*Picea abies*) is traditionally used in folk medicine to heal skin ulcers and infected wounds. Its antimicrobial properties were studied against certain human bacteria important in infected skin wounds. The sensitivity of the resin against Gram-positive and Gram-negative bacteria was studied *in vitro* by methods that are routinely used in microbiology laboratories. The resin salve exhibited a bacteriostatic effect against all tested Gram-positive bacteria but only against *Proteus vulgaris* of the Gram-negative bacteria. Interestingly, the resin inhibited the growth of bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococcus (VRE), both on agar plates and in culture media. The study demonstrated antimicrobial activity of the resin salve and provided objective evidence of its antimicrobial properties. It gives some explanations why the traditional use of home-made resin salve from Norway spruce is experienced as being effective in the treatment of infected skin ulcers.

Key words: Resin; Norway spruce; microbiology; MRSA; VRE; skin ulcer; infection.

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The emergence of multidrug resistance of human pathogenic bacteria and many undesirable adverse effects of antibiotics has led to a search for new antimicrobial agents also of plant origin. Plants produce antimicrobial compounds (1–10).

Resin excretion obviously provides trees with protection against bacterial and fungal infections (10). Various plant products, wood tar and resins exhibit antimicrobial effects against human bacteria, and might therefore become tools to treat human infections (11–17). In some earlier experiments these compounds, e.g., resin acids, have antimicrobial effects against human bacteria as well as toxic effects against aquatic organisms. The concentrations often need to be high and this may cause irritation (18).

Home-made resin salve from Norway spruce (*Picea abies* (L) Karsten) (19) used to heal skin wounds and various skin infections is an example of centuries-long folk medicine in Lapland, Northern Finland. The resin salve is prepared by boiling resin with butter or other animal fat, and—among lay people—has the reputation of being effective. Our own empirical

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observations support this and suggest that the resin salve may be a therapeutically and clinically useful tool in infected wounds (17). There are some scientific reports that have shown that the resin-derived products may be potential tools for treating skin infections and infected wounds (13). However, home-made resin salve has received a little attention in modern medicine and the antibacterial effects of these resin salves, especially against clinically important microorganisms in humans, are unknown. In this study we tested *in vitro* the antibacterial effects of spruce resin and resin salve against some clinically important species of bacteria, including drug-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococcus (VRE).

MATERIAL AND METHODS

Preparation of spruce resin

The resin was collected in Kolari, Finnish Lapland, from trunks of full-grown Norway spruce with knives by permission of the landowners. Bark and other impurities were removed from the resin by mechanical cleaning and the resin was stored in a refrigerator (+4 °C) until further processed. To make the resin grease according to traditional procedures, it was mixed and boiled (around +100 °C) with salt-free ordinary butter (Valio Ltd., Helsinki, Finland) by stirring, in a weight proportion of 1:3 (w/w). After cooling, the resin salve was packed in clean glass bottles, or in salve tubes, and was kept in a refrigerator until used. MIC values were determined with pure spruce resin. The resin was filtered through a fabric at 90 °C to eliminate impurities. After filtration the resin was frozen at −20 °C and pulverized in a mortar.

Culture media

Agar diffusion tests. The experiments were performed by slight modifications of procedures usually applied in tests of antimicrobial effects of antibiotics against bacteria (20–22). Muller-Hinton medium was chosen as agar according to clinical laboratory standards (22). Using Mueller-Hinton agar plates, wells with a diameter of 8 mm were punched into the agar medium and filled with the resin salve. The plates were incubated at 35 °C in ambient air for 18 h before measuring zones of inhibition. Also, drops of resin salve (approximately 0.5 ml; 500 mg) were placed on agar without punched wells. The experiments were continued by dropping sterile saline on the resin salve preloaded on the agar plate. Drops and wells filled with sterile saline without resin salve were used as controls.

Because of the small inhibition zones in agar diffusion test, agar dilution tests and liquid media, experiments were performed as described below as more sensitive and illustrative methods.

Agar dilution tests. The MIC plates were prepared by slightly modifying a method used in essential oil research (1, 2, 20). Mueller-Hinton (Scharlau, Barcelona, Spain) agar was prepared according to the manufacturer's instructions. Tween 80 (Fluka Ag, Buchs, Switzerland) was added to Mueller-Hinton media to give a concentration of 1.0% (w/v) with the addition of 1.0% (w/v). Pulverized spruce resin was added to 500 ml aliquots of liquid agar to give final concentrations of 1.0, 0.8, 0.6, 0.4 and 0.2% (w/v) in the media. The media/resin solutions were autoclaved at 121 °C for 15 min and poured into Petri dishes. The effect of autoclaving on resin antimicrobial activity was investigated by inoculating the Mueller-Hinton plate with *S. aureus* and applying the agar-well method for autoclaved and non-autoclaved spruce resin, as explained above.

The bacteria used in the MIC experiments were grown overnight at 35 °C on Mueller-Hinton agar. Bacterial biomass was suspended to 0.9% NaCl solution and adjusted to match the 0.5 McFarland turbidity standard. Three 1 µl aliquots of the bacterial suspensions were delivered onto the surface of three parallel Mueller-Hinton/resin plates using a calibrated loop. Inoculated plates were incubated at 35 °C. Bacterial growth was inspected after 16–20 h. The plates which did not show any bacterial growth at 1% (w/v) spruce resin concentration after 16–20 h were incubated for 5 days.

Liquid media experiments. To test the effect of resin on selected bacteria, the strains tested were cultivated in liquid media with and without resin. The liquid growth medium used was fastidious anaerobe broth (FAB) (Lab M Ltd., Bury, England). The medium is rich in nutrients and includes 0.75 g/l agar. Due to bacterial growth, the broth becomes turbid or—depending on the bacteria tested—visible colonies are formed in the medium. FAB medium was used because of its semisolid property, which made it possible to directly observe the number of colonies of some bacteria after incubation. Liquid media experiment with FAB was also the most sensitive method; it brought out the difference between *Proteus vulgaris* and *Proteus mirabilis.*

The experiments with the FAB medium were performed in two different ways. First, the tested bacteria were inoculated into 1 ml FAB medium with or without a drop (0.05 ml) of resin salve. In the FAB tubes, colonization of the bacteria was examined by turbidity of the growth medium and recorded as absent, mild, moderate, or heavy. Secondly, FAB media with or without pretreatment with resin salve were prepared. In preparing the resin-pretreated FABmedium, a layer of resin salve was spread on the bottom of a Petri dish, after which 12 ml of FAB solution

was layered on this resin bed. The plate was covered by parafilm and incubated at room temperature for various time periods (from 15 min to 72 h). After incubation, the FAB medium was removed from the plate with a Pasteur pipette. The FAB media with or without pretreatment with resin were used in incubation experiments with various bacteria, as described below. After pretreatment with resin, the FAB medium was clear and did not differ macroscopically from the FAB medium that was not pretreated with resin. However, the pH of the medium was slightly lower after pretreatment (6.5 *vs* 7.6).

The inoculum of bacteria to be tested was prepared by making a saline suspension of isolated colonies selected from an 18- to 24-h blood agar plate. The suspension was adjusted to match the 0.5 McFarland turbidity standard and serially diluted (10-fold) from 10^{-1} to 10^{-6}. An inoculum of 100 µl of undiluted suspension and appropriate dilutions were added to each tube with 900 µl of medium.

Reference (control) cultures were inoculated in tubes without resin, or without pretreatment of the test medium with resin (see above). The tubes were incubated at 35±2°C for 18 h for the first inspection and further for up to 72 h.

Growth of bacteria in test tubes was visually monitored under adequate light and photographed as needed. Antibacterial activity was detected as decreased turbidity in cultures containing resin as compared to those without resin. After liquid culture tests, viability of bacteria was also tested by cultivation of grafts (samples) on blood agar plates. Subcultures on agar plates were performed in order to determine whether the resin killed the bacteria or only inhibited their growth (bacteriocidic *vs* bacteriostatic).

The bacteria used in the experiments are listed in Table 1.

RESULTS

The bacteria grew well in FAB media in the absence of resin (Figs. 1 & 2). The antibacterial activity of resin, expressed arbitrarily as decreased turbidity *vs* control, is presented in Table 1. Butter alone had no antimicrobial effect.

The antimicrobial effect was clearly higher against Gram-positive organisms than against Gram-negative organisms, with the exception of *P. vulgaris* (Table 1).

Dilution of the resin-pretreated FAB medium with untreated FAB medium at a ratio of up to 1:2 was sufficient to totally inhibit the bacterial growth, even at the highest concentration of bacteria in the inoculum. Inhibition disappeared at dilutions of 1:5, or more. The inhibition persisted for several days (tested up to 3 days). Subcultures from the resin-FAB medium onto the blood agar plate still 3 days after inoculation showed regrowth of the bacteria, sug-

TABLE 1. *Growth of bacteria in FAB medium with and without presence of (or pretreatment with) spruce resin and minimum inhibitory concentrations (MIC) of spruce resin in Mueller-Hinton agar after 16–20 h incubation*

	FAB	Resin-FAB	MIC (%,w/v)	Growth in 1% (w/v) resin concentration after 5 days
Gram-positive cocci				
Staphylococcus aureus ATCC 25923	+++	−	0.4	NO
Staphylococcus aureus (MRSA) NEQAS 4937/98	+++	−	0.4	NO
Staphylococcus epidermidis ATCC 49461	+++	−	0.4	NO
Enterococcus faecalis ATCC 29212	+++	−	0.6	YES
Enterococcus faecalis (VRE) (vanB) EARSS UA605/01	+++	−	0.4	YES
Enterococcus faecalis EARSS UA1527/01	+++	−	0.4	YES
Streptococcus pyogenes (A) ATCC 19615	+++	−	0.2	NO
Streptococcus agalactiae (B) NEQAS 6098/01	+++	−	0.2	NO
Gram-positive rods				
Arcanobacterium haemolyticum LABQ 237/95	+	−	N.A.	N.A.
Gram-negative rods				
Escherichia coli ATCC 25922	+++	+++	>1.0	
Enterobacter cloacae ATCC 23355	+++	++	>1.0	
Klebsiella pneumoniae ATCC 13883	+++	+++	>1.0	
Proteus vulgaris ATCC 8427	+++	−	>1.0	
Proteus mirabilis ATCC 12453	+++	+++	>1.0	
Pseudomonas aeruginosa ATCC 27853	+++	+++	>1.0	

− No visible growth. + Slight growth. ++ Moderate growth. +++ Heavy growth. N.A. Not analyzed.

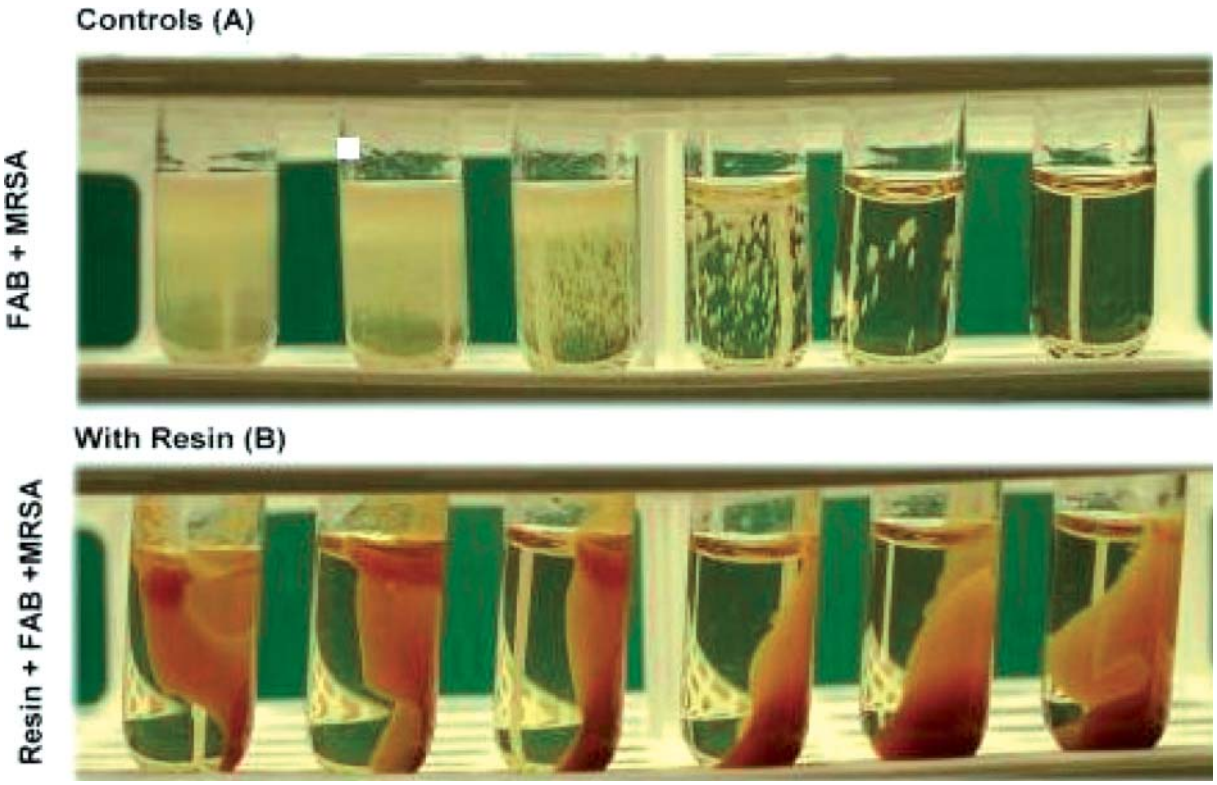

Fig. 1. Inhibition of growth of *MRSA* in FAB medium pretreated with resin (resin-FAB+MRSA); i.e., before inoculation of *MRSA* the FAB medium was pretreated for 1 h with resin (see Materials and Methods). FAB+*MRSA* indicates tubes without pretreatment with resin.

gesting that the resin inhibited growth rather than killing the organisms, i.e., the resin is bacteriostatic rather than bacteriosidic.

A drop of resin salve (volume approximately 0.5 ml) (Fig. 3) showed a narrow but clearly visible inhibition zone on Petri plates with susceptible bacteria. Adding sterile saline on the resin salve on the agar plate caused inhibition of the bacterial growth in the whole area where saline was spread (Fig. 3).

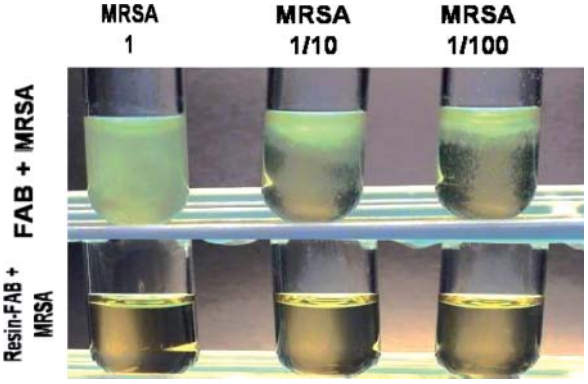

Fig. 2. Inhibition of the growth of *MRSA* in FAB medium pretreated with resin (resin-FAB+MRSA). Before inoculation of *MRSA* the FAB medium was pretreated for 1 h with resin (see Materials and Methods). FAB+*MRSA* indicates tubes without pretreatment with resin.

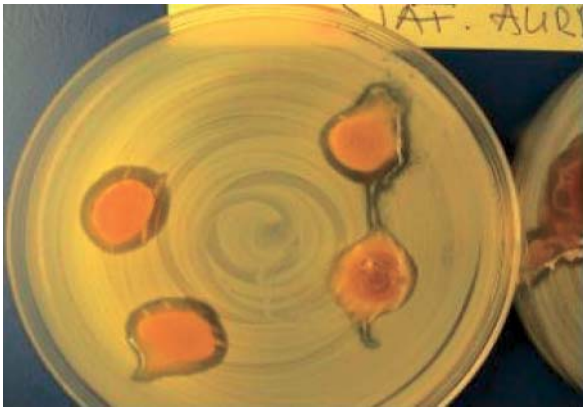

Fig. 3. Sterile saline was added on resin drops on the plate. Note inhibition of bacterial growth in area where saline was spread.

Autoclaving did not have any effect on the antimicrobial activity of spruce resin against *S. aureus.* The growth inhibition zones around the autoclaved and non-autoclaved resin were narrow and identical. The resin itself did not show any growth of bacteria or fungi when tested in various growth media (blood agar, chocolate agar, Sabouraud-dextrose agar, FAB medium). Nor was there any microbial growth when resin had been stored in the refrigerator or at room temperature for more than 2 years.

MIC values of the spruce resin were lowest for bacteria of the genus *Streptococcus*, followed by *Staphylococcus* and *Enterococcus* (Table 1). *Enterococcus* strains still grew at 1% (w/v) spruce resin concentration after 5 days of incubation. The MIC values of Gram-negative bacteria were over 1% (w/v), including *P. vulgaris.*

DISCUSSION

This study provides convincing evidence that home-made resin salve of Norway spruce (*Picea abies* (L) Karsten (19)) has antimicrobial effects against clinically important Gram-positive bacteria. An exception among the Gram-negative bacteria was *P. vulgaris,* the growth of which was also clearly inhibited by the resin salve, although the MIC value of pure spruce resin for *P. vulgaris* exceeded 1.0% (w/v) as for other Gram-negative bacteria. The reason for this discrepancy is probably the higher concentration of spruce resin in the resin salve (25% w/w) as compared to the highest concentration of spruce resin (1% w/v) for the MIC determinations.

The tests suggest that the antimicrobial effect is bacteriostatic rather than bacteriocidic, since there was regrowth of bacteria in grafts (samples) from FAB medium on agar plate even after 3 days of incubation of bacteria. A clear indication of a bacteriostatic effect was also the growth of *Enterococcus* strains at 1% (w/v) resin concentration after 5 days of incubation, although the MIC values for *Enterococcus* strains after 16–20 h incubation were 0.4–0.6% (w/v).

Interestingly and importantly, spruce resin was antimicrobial against *VRE* and all *staphylococci* tested, including *MRSA.* A similar anti-MRSA effect has been reported for resin acids (isopimaric acid) from pine (*Pinus nigra*) resin (19). The observation of a broad antimicrobial effect of spruce resin salve against Gram-positive bacteria—the key pathogens of wound infections—may be of clinical importance and may partly explain why treatment with Lappish traditional resin therapy heals severe, deep chronic skin ulcers (17). However, clinical observations on the efficacy of resin treatment in skin ulcers are so far only empirical. Nevertheless, the present microbiological observations and previous experience make a strong case for conducting a prospective, randomized and controlled trial on the effect of spruce resin in severe (stage II–IV) pressure ulcers. Such a trial is, in fact, already in progress.

The mechanisms of the antimicrobial effect of spruce resin are poorly known (11, 12, 23–25). The mechanisms of the resin seem, however, to be "specific" since the resin salve inhibits the growth of Gram-positive bacteria but not the growth of all bacteria. Resin acids (terpenes) have been observed to destroy the cellular integrity of yeast cell mitochondria (24, 25). The antimicrobial effect of the resin could—at least partially—be explained by this membrane-damaging activity. In such cases the outer membrane of Gram-negative bacteria may act as a protective barrier against antimicrobial compounds, as seems to be the case for essential oils obtained from plant material (20).

The present observations indicate that this influence may be mediated by water soluble substance(s) although the resin itself is largely water insoluble, since pretreatment of FAB medium with resin transferred the antimicrobial influence from the resin to the FAB medium. In addition, experiments on agar plates, in which a drop of sterile saline on resin immediately spread the antibacterial effect over the whole area, support this hypothesis.

Our preliminary experiments (unpublished data) with abietic acid show striking similarities of antimicrobial (and antifungal) effects with the resin salve, indicating that resin acids may be the therapeutically effective antimicrobial components of the home-made resin salve. However, the role of abietic acid and of other resin acids in the antimicrobial effects requires additional studies.

In conclusion, our study shows that traditional Lappish resin salve has a very important antimicrobial influence on the growth of pathogenic skin bacteria, including *MRSA* and

VRE. These findings demonstrate the necessity for further clinical studies on the usefulness of resin-based regimens in treating severe wound infections.

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