APPLIED MICROBIOLOGY, Jan. 1972, p. 130-134
Vol. 23, No. 1

Printed in U.S.A.

Reproducible Topical Staphylococcal Infection in Rats[1]

FAITH GOLDSCHMIDT

Johnson and Johnson Research, North Brunswick, New Jersey 08903

Received for publication 26 July 1971

A topical infection model for the study of the effectiveness of antimicrobials was developed. Animals were laparotomized, sutured with braided silk, and inoculated with a strain of *Staphylococcus aureus*. The test organism was phage typed, and its antibiotic spectrum was determined. Concentrations of bacteria from 5×10^4 to 10^8 cells per incision produced large body wall stitch abscesses with occasional drainage through the skin. This laparotomy infection is readily reproducible and can be used for evaluation of the ability of topical antimicrobials to prevent *S. aureus* stitch abscesses.

The evaluation of topical antimicrobials has been hampered by lack of a suitable infection model. Infections have been induced in humans (6) and in animals. The types of wounds used for studying animal-induced infections include burns (13), strip wounds (5), and incisions (10, 15, 16, 19). Techniques used to induce infections may involve increased physical trauma to the wound. Physical trauma may be induced by foreign object implantation (1-3, 11, 12, 17), extra needle tracts, devitalized tissue, or pursued sutures (7-9, 14). None of these experimental models parallels closely the course of human accidental infections.

A method for creating a reproducible *Staphylococcus aureus* infection in topical incisions in rats has been developed. The method does not require implantation of extra foreign objects or the excessive trauma or artificial lowering of resistance of test animals described by other investigators, and it parallels the human postoperative infection process.

MATERIALS AND METHODS

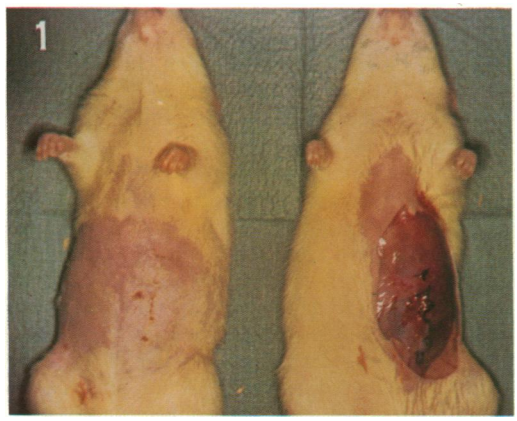
Test organism. A strain of *S. aureus* designated 1R was isolated from a conventional rat. It was untypable with human phage. This organism was resistant to penicillin and terramycin but sensitive to aureomycin, bacitracin, chloramphenicol, dihydrostreptomycin, erythromycin, neomycin, polymyxin B, and tetracycline. It contained free coagulase, but not bound coagulase, and was nonencapsulated (4, 18, 20). The culture was transferred every 18 to 24 hr in Trypticase soy broth (TSB). Plate counts were made daily to establish approximate numbers present in the culture. On the day of inoculation the culture was diluted in 0.1% sterile peptone-water to the desired dose level. Plate counts were done to determine the exact dose. Numbers of viable cells used as inocula ranged from 5×10^2 to 10^8 per incision.

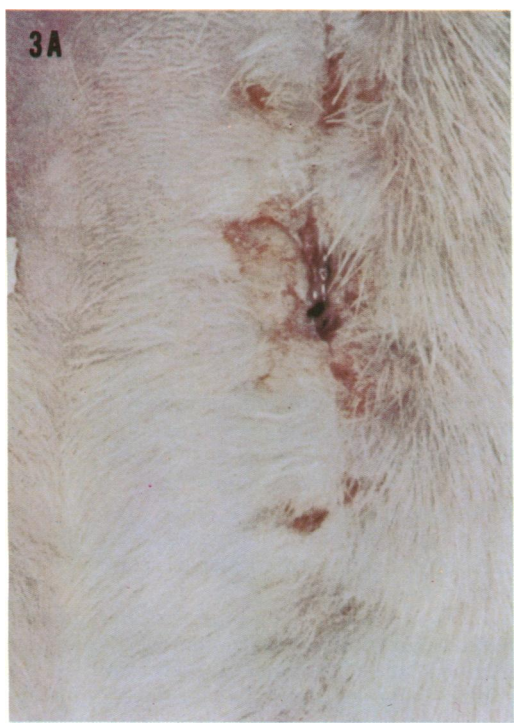
Test animals. Male, 200-g MW-3 Wistar-derived specific pathogen-free rats from Manor Research South were used for all infection studies. The animals were received in filter shipping boxes and were individually housed in Isosystem cages (Carworth Labs). Disposable water bottles, Purina Rat Chow, tap water, and conventional bedding were used. Stoppers, spouts, and metal cage tops were autoclaved and washed between experiments. Personnel wore sterile gloves and operating room gowns whenever handling animals.

Test methods: environmental sampling. Organisms normally present in the animal room and in and on the test animals were monitored so that the source of any spontaneous infections could be determined. Air was sampled with an Andersen air sampler containing blood-agar (BA) plates. Surfaces were sampled by using weighed amounts of sample or by swabbing. The normal flora of rats were determined. Nose, skin, and mouth areas were swabbed. and 1-g samples of fresh intestinal contents were weighed. Samples were diluted and plated in brain heart infusion agar (BHI) for total counts and on differential media for types of organisms.

Operative technique. Animals were anesthetized with an intraperitoneal injection of 36 mg of chloral hydrate (Merck) per 100 g of body weight. The abdomen was shaved, and an incision about 6 cm long was made with a razor blade through the skin to the left of midline. The connective membranes were cleared from between the skin and body wall with blunt-sharp scissors. Sharp-sharp scissors were then used to make a 6-cm incision through the exposed body wall. The laparotomy was sutured in two layers. Body wall was sutured with 4-0 braided silk,

[1] Presented in part at the 70th Annual Meeting of the American Society for Microbiology, Boston, Mass., 26 April-1 May 1970.





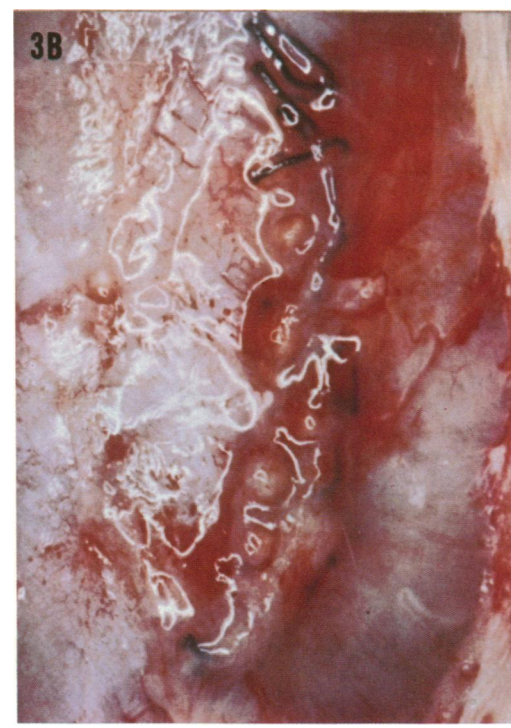


FIG. 1. *Skin and body wall of rats inoculated with NaCl or peptone-water.*
FIG. 2. *Draining skin of rat inoculated with 10^8 cells of 1R.*
FIG. 3. *Skin and body wall of rats inoculated with 5×10^5 cells of 1R.*

and 000 braided silk was used for the skin. Interrupted stitches were placed about 0.5 cm apart.

Sampling technique. Animals were sacrificed at various time periods from 1 hr to 14 days. They were anesthetized with chloral hydrate. The surface of the incision was swabbed with a wet swab which was then placed in 10 ml of peptone-water. One milliliter of blood was withdrawn from the heart and placed in 9 ml of peptone-water. The skin, including the sutured incision, was separated and removed. Body wall tissue, including the incision, was also removed. One-gram samples of both tissues were weighed out and ground in peptone water in a tissue homogenizer for 2 min. The supernatant fluids were diluted and plated. The skin swab and blood samples were also diluted, and portions of each dilution were plated in BHI agar and on differential media. Any *S. aureus* cells recovered were tested for antibiotic spectrum as a means of identification of infecting strain.

Test for spontaneous infections. Animals were laparotomized without deliberate contamination. Subsequent to suturing, 0.1 ml of sterile NaCl or peptone-water was placed on top of the sutured skin. The inoculum was rubbed into each incision with the side of the delivery needle.

Artificial infections. Animals were inoculated after suturing with 0.1 ml of suspension of *S. aureus* containing known numbers of viable cells. The inoculum was rubbed into the wound. NaCl or peptone-water controls were included in these studies.

RESULTS AND DISCUSSION

Environmental sampling. The normal flora of specific pathogen-free rats included *Streptococcus mitis*, coagulase-negative staphylococci, enterococci, gram-positive bacilli, *Enterobacteriaceae*, and molds. The numbers recovered from nose ranged from 10^2 to 2×10^3 per sample; from skin, 10^2 to 10^3 per sample; from mouth, 10^4 to 10^6 per sample; and from feces, 10^8 to 3×10^9 per g. *S. aureus* was not isolated from these rats.

Air and surfaces were contaminated with molds, *Bacillus* sp., *Streptococcus* sp., coagulase-negative staphylcocci, and yeast. Molds, *Enterobacteriaceae*, *Bacillus* sp., coagulase-negative staphylococci, and yeast were isolated from food, bedding, cages, bottles, and water. The ranges of numbers of organisms recovered are shown in Table 1.

TABLE 1. *Microbial contamination of environment*

Site of sample	No. of organisms
Air	2–6 per ft^3
Surfaces	0–12 per plate
Water	$<10^6$–3×10^6 per ml
Bedding	<50–6×10^4 per g
Food	2×10^2–8×10^4 per g
Cages	<5–2×10^3 per 16 cm^2
Water bottles	<3–2×10^6 per 16 cm^2
Water-bottle stoppers	3×10^3–4×10^5 per sample
Drinking tubes	<3–7×10^6 per sample

Test for spontaneous infections. Laparotomy wounds treated with NaCl or peptone-water healed in 9 to 10 days (Fig. 1). No evidence of infection was observed in a total of 126 animals. The organisms recovered at the levels shown in Table 2 were all normal flora.

Artificial infections. The appearance of the incisions of rats contaminated with *S. aureus* depended on the dosage of organisms. An inoculum of 5×10^2 cells per incision produced body wall stitch abscesses which healed in 9 to 10 days (11 rats). An inoculum of 5×10^3 cells also produced stitch abscesses, but healing did not occur in all cases (12 rats).

Animals which received 5×10^4 to 10^8 cells developed abscesses which did not heal within 19 days (196 rats). An inoculum of 5×10^6 or more cells per incision produced draining body-wall abscesses and necrotic skin in 35 rats (Fig. 2). Occasionally skin and body wall were adhered. An average inoculation number of 5×10^5 cells per incision (range, 5×10^4 to 10^6) was selected as optimal for subsequent experiments. All 161 rats contaminated with this range of organisms showed consistent, localized, intact body wall abscesses (Fig. 3B) and occasional skin necrosis (Fig. 3A). These symptoms appeared from 4 to 7 days after surgery and persisted through 19 days. The general health of the animals was good. No organisms were recovered from heart blood, indicating that the infection was not systemic.

Smears of the incision surface showed mixed normal flora and *S. aureus*. Numbers fluctuated but tended to drop to normal levels in 6 to 7 days. Pure cultures of *S. aureus* 1R were recovered from skin and body wall tissue samples of rats contaminated with 5×10^3 or more cells.

Numbers of organisms recovered from full-thickness skin and body wall samples from 1 hr to 14 days were consistently higher in artificially contaminated wounds than in control wounds. Figures 4 and 5 show, respectively, the comparison of contaminated versus control skin and contaminated versus control body wall from 1 to 48 hr.

Figures 6 and 7 show numbers in contaminated skin tissue versus control tissue and numbers in contaminated body wall versus control body wall from 1 to 14 days. Numbers in skin tissue dropped at 7 days postoperation. In control animals, this could be due to healing of the wound. Closure prevented external organisms from entering the wound, and host de-

TABLE 2. *Average total numbers of uninfected laparotomies per day*[a]

Day	Skin smear	Skin tissue per g	Body wall tissue per g
1	6×10^3	4×10^5	5×10^1
2	6×10^5	10^6	10^4
3	5×10^2	8×10^5	8×10^2
4	5×10^3	4×10^6	5×10^2
5	3×10^3	6×10^4	
6	10^4	5×10^5	5×10^2
7	10^4	10^6	8×10^2
14	10^5	10^4	9×10^3

[a] In brain heart infusion agar.

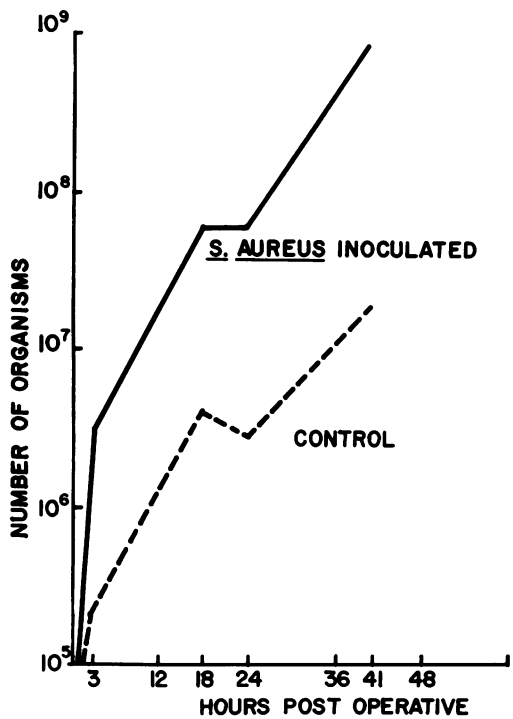


FIG. 4. *Average number of bacteria per gram of skin per hour.*

fense mechanisms cleared those contaminating bacteria already present. With no recontamination, the total numbers within skin tissue dropped. This could be true for the artificially infected animals, but the drop in numbers in skin tissue and also the increase in body-wall numbers at day 7 to 10 (Fig. 7) could be due to migration of 1R to the body wall and localization within walled abscesses. *S. aureus* remained viable within abscesses.

For a stable, reproducible infection to occur with the described *S. aureus* strain, more than 5×10^3 cells per incision and pure colonization

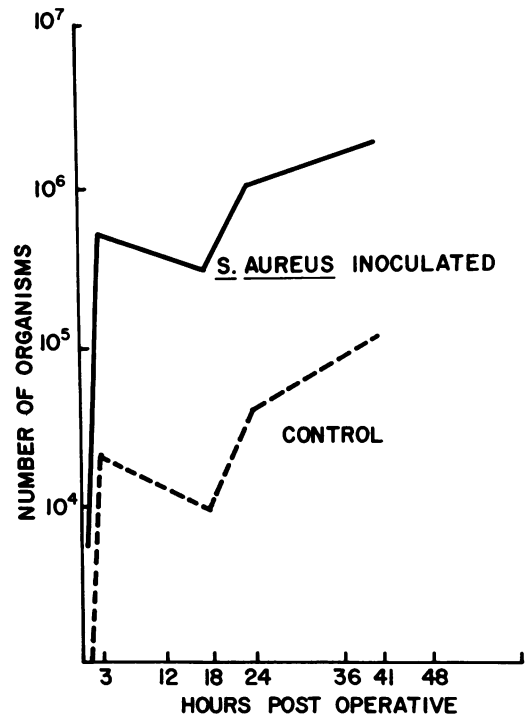


FIG. 5. *Average number of bacteria per gram of body wall samples per hour.*

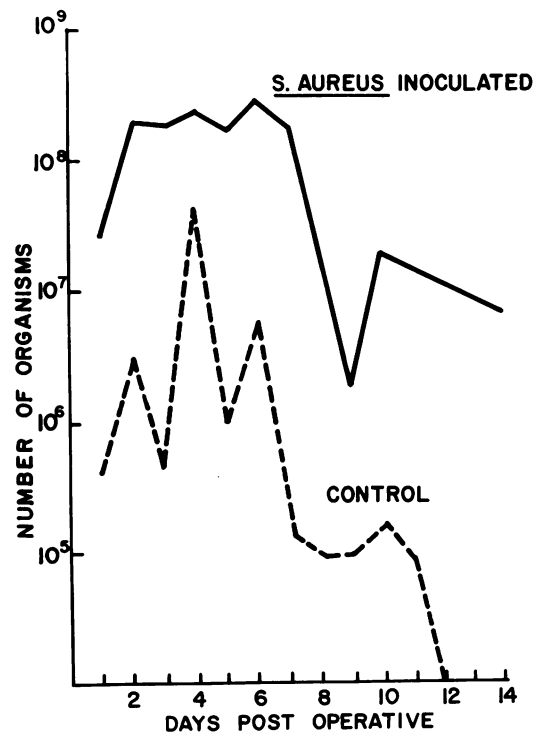


FIG. 6. *Average number of bacteria per gram of skin from 1 to 14 days postoperation.*

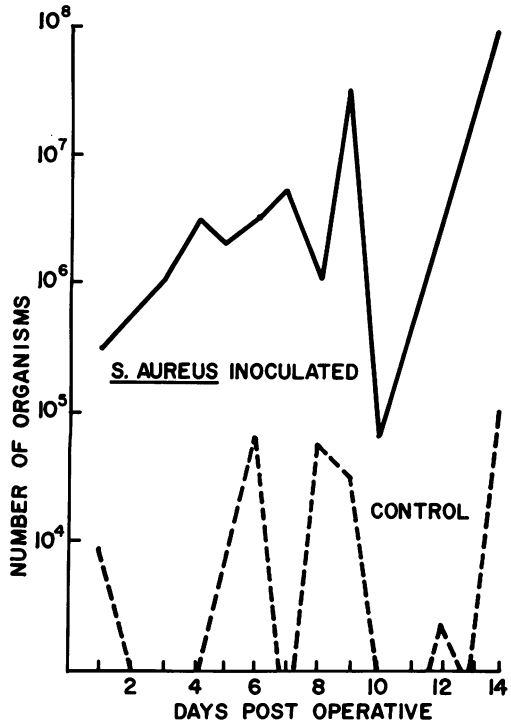


FIG. 7. *Average number of bacteria per gram of body wall from 1 to 14 days postoperation.*

by the test organism were needed. This infection model is suitable for evaluating the effectiveness of topical antimicrobial agents against this organism. Such evaluation is now in progress, and results will be reported in subsequent papers.

ACKNOWLEDGMENTS

I am grateful to Terezia Laszlo, Donna Murasko, Shirley Kerber, and Linda Williams for their technical assistance, and also to Betty J. Means for her ideas and encouragement.

LITERATURE CITED

1. Agarwal, D. S. 1967. Subcutaneous staphylococcal infection in mice. I. The role of cotton dust in enhancing infection. Brit. J. Exp. Pathol. **48**:436-449.
2. Agarwal, D. S. 1967. Subcutaneous staphylococcal infection in mice. II. The inflammatory response to different strains of staphylococci and micrococci. **48**:468-482.
3. Agarwal, D. S. 1967. Subcutaneous staphylococcal infection infection in mice. III. The effect of active and passive immunization and anti-inflammatory drugs. **48**:483-500.
4. Alami, S. Y., and F. C. Kelly. 1960. Influence of coagulase and route of infection on staphylococcal virulence in mice. Proc. Soc. Exp. Biol. Med. **105**:589-591.
5. Burnett, J. W. 1963. Trauma in experimental superficial cutaneous infections. Arch Dermatol. **88**:276-279.
6. Duncan, W. C., et al. 1970. Experimental production of infections in humans. J. Invest. Dermatol. **54**:319-323.
7. Edlich, R. F., M. S. Tsung, W. Rogers, P. Rogers, and O. H. Wangensteen. 1968. Studies in the management of the contaminated wound. I. Technique of closure of such wounds together with a note on a reproducible experimental model. J. Surg. Res. **8**:585-592.
8. Edlich, R. F., W. Rogers, G. Kasper, D. Kaufman, M. S. Tsung, and O. H. Wangensteen. 1969. Studies in the management of the contaminated wound. I. Optimal time for closure of contaminated open wounds. II. Comparison of resistance to infection of open and closed wounds during healing. Amer. J. Surg. **117**:323-329.
9. Edlich, R. F., J. Custer, J. Madden, A. S. Dajani, W. Rogers, and O. H. Wangensteen. 1969. Studies in the management of the contaminated wound. III. Assessment of the effectiveness of irrigation with antiseptic agents. Amer. J. Surg. **118**:21-30.
10. Glotzer, D. J., W. S. Goodman, and L. H. Genonimus. 1970. Topical antibiotic prophylaxis in contaminated wounds. Arch. Surg. **100**:589-593.
11. Hunt, T. K., E. Jawetz, J. G. P. Hutchison, and J. E. Dunphy. 1967. A new model for the study of wound infection. J. Trauma **7**:298-306.
12. James, R. C., and C. M. MacLeod. 1961. Introduction of staphylococcal infections in mice with small inocula introduced on sutures. Brit. J. Exp. Pathol. **42**:266-277.
13. Millican, R. C. Infection in delayed deaths. 1962. *In* C. P. Artz (ed.), Research in burns. Proc. First Int. Cong. Res. Burns. F. A. Davis Co., Philadelphia.
14. Miraglia, G. J. 1970. Model infections in the evaluation of antimicrobial agents. Trans. N.Y. Acad. Sci. **32**:337-347.
15. Möller, A., and B. Rydberg. 1969. Influence of a cationic detergent on the development of infection in experimental wounds contaminated with staphylococci. Acta. Chir. Scand. **135**:459-465.
16. Noble, W. C. 1965. Staphylococcal subcutaneous skin lesions. Brit. J. Exp. Pathol. **46**:254-262.
17. Russell, H. E., D. P. Gutekunst, and R. E. Chamberlain. 1968. Evaluation of furazolium chloride in topical treatment of model infections in laboratory animals. Antimicrob. Ag. Chemother. 1967, p. 497-502.
18. Smith, D. T. 1968. The Staphylococci, p. 447-473. *In* D. T. Smith, C. F. Conant, and H. P. Willett (ed.), Zinsser microbiology, 14th ed. Appleton-Century Crofts, New York.
19. Smith, M., and I. E. Enquist. 1967. A quantitative study of impaired healing resulting from infection. Surg. Gynecol. Obstet. Int. Abstr. Surg. **125**:965-973.
20. Tompsett, R. 1962. Role of phagocytosis in immunity to clumping factor-negative staphylococci. Antimicrob. Ag. Chemother. 1962, p. 100-106.