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Editorial

Contents

Well to Jump start with this issue we have the 'Mini Review' section giving us a brief on Elementary identification for Enterobacteriaceae based on few basic biochemical tests. Enteric pathogens such as *Salmonella* species should be identified biochemically and typed serologically. *Hafnia, Morganella* and *Proteus* species can resemble non-motile *Salmonella* biochemically, and can agglutinate in polyvalent *Salmonella* antisera. Because of such diversity of biochemical activities, all the reactions of every species are not described, therefore only a few screening tests which are performed for the clinical microorganisms are included together with results for the more common genera and species, in this article.

Our Current Trends section highlights about the results of two clinical studies designed to determine the antimicrobial effectiveness of CHG/ethanol-emollient hand preparation using the log reduction criteria for bacterial counts on the hands defined by the Food & Drug Administration's (FDA) Tentative Final Monograph for Health-Care Antiseptic Drug Products (TFM).

In Profile Scientist – **Venkatraman "Venki" Ramakrishnan** was born in 1952 is an American and British structural biologist of Indian origin. In 2009 he shared the Nobel Prize in Chemistry with Thomas A. Steitz and Ada Yonath, "for studies of the structure and function of the ribosome.

Bug of the month - *Yersinia pestis* was discovered in Hong Kong in 1894 by a Swiss physician Alexandre Yersin. *Yersinia pestis* is a rod shaped gram-negative bacteria that can also have a spherical shape. *Y. pestis* causes diseases through the bite of an infected rat or flea, but can also be transmitted by air.

Did You Know? A team of scientists designed a device that can induce partial hind limb regeneration in adult aquatic African clawed frogs (*Xenopuslaevis*) by "kick-starting" tissue repair at the amputation site. The procedure induced a regenerative response they normally never have, which resulted in bigger, more structured appendages. The bioreactor device triggered very complex downstream outcomes that bioengineers cannot yet micromanage directly."

Best Practices - Burn care is conducted by members of a multidisciplinary burn team which include medical, surgical, intensive care, nursing, physiotherapy, occupational therapy, dietetics, social work, psychiatry, psychology, speech therapy, pharmacy and technicians. A multidisciplinary approach to burn management is essential for optimal functional and cosmetic outcome.

Get inspired by the motivational thoughts in our relaxed mood section.

Our JHS team is thankful to all our readers for their immense support. Feedback & suggestions are always welcomed.

Bacteriology - Elementary Identification of Enterobacteriaceae (Issue 2)

The identification of medically important genera of the family Enterobacteriaceae was discussed in previous issue. In this article, elementary identification for Enterobacteriaceae based on few basic biochemical tests will be briefed.

Enteric pathogens such as Salmonella species should be identified biochemically and typed serologically. *Hafnia, Morganella* and *Proteus* species can resemble non-motile *Salmonella* biochemically, and can agglutinate in polyvalent *Salmonella* antisera. Because of such diversity of biochemical activities, all the reactions of every species are not described, therefore only a few screening tests which are performed for the clinical microorganisms are included together with results for the more common genera and species, in this article.

Principles of Identification

In medical laboratories, routine practice for elementary identification of bacterial microorganisms comprises of colonial morphology, Gram stain, oxidase test and the use of several biochemical tests are carried out to identify isolates from clinical material.

Careful consideration should be given to isolates that give an unusual identification. All evidence including growth characteristics, cultural morphology and serology should be considered before accepting commercial identification system results.

Full molecular identification using for example, Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) can be used to identify isolates to species level. If further identification or confirmation is required, isolates should be sent to the Reference Laboratory.

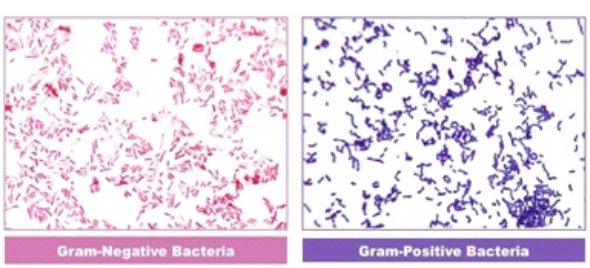
Bacterial pathogens associated with Human Infections:

Citrobacter gillenii (Formerly *Citrobacter Genomospecies*), *Citrobacter murliniae* (Formerly *Citrobacter Genomospecies*), *Leminorella grimontii, Leminorella richardii, Moellerella wisconsensis,* though, other genera and species of the Enterobacteriaceae may rarely be associated with human disease.

Identification

Microscopic Appearance

Gram stain is basic microbiological staining procedure used to differentiate the gram character of the bacterial pathogens, based on the structure and on the color of the bacterial cells observed microscopically. The Gram negative bacteria may show short rods, long rods and are stained pink, while, Gram positive bacteria appear round or spherical cocci shaped and are stained blue-purple. However, few Gram negative rods may also show bipolar staining (eg. *Yersinia* species).

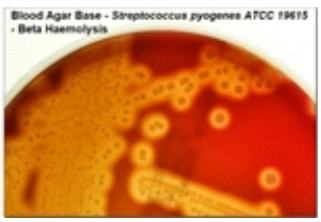


Primary Isolation Media

Culture media routinely used for the primary isolation of the pathogenic bacteria from clinical samples are Blood agar, MacConkey agar, Cystine-lactose-electrolyte deficient (C.L.E.D.) agar with bromothymol blue or Andrade's indicator, and also Chromogenic media which are available depending on specimen type or specific target organism. These media are inoculated and incubated in air at 35 - 37°C for 18 - 24hr.

Colonial Appearance on Primary Isolation Media

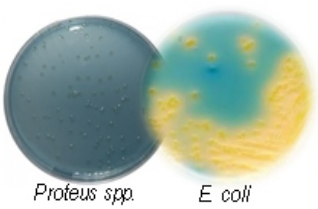
On Blood agar (Blood Agar Base – AM1014/ AM5014), the pathogenic bacterial colonies are 2 - 3mm diameter, low, convex, grey, smooth or mucoid, may be haemolytic or swarming.



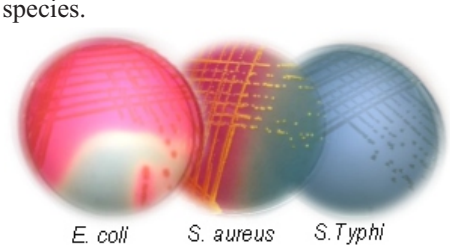
Streptococcus pyogenes

On MacConkey agar (AM1059/ AM5059), the pathogenic colonies may appear pink (lactose fermenting) or colourless (lactose non-fermenting), size and shape vary with individual species.

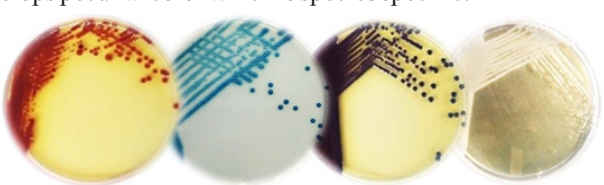
On C.L.E.D. agar with bromothymol blue (AM1027/ AM5027), the colonies may appear yellow (lactose fermenting) or blue (lactose non-fermenting), size and shape vary with individual species.



On C.L.E.D. agar with Andrade's indicator (AM1026 / AM5026) colonies may appear pink (lactose fermenting) or green, translucent (lactose non-fermenting), size and shape vary with individual species.



For colonial appearance of specific target organism on chromogenic media available as Michrom UTI Agar (AM1069277 / AM5069277), depends on chromogenic reaction between the particular substrate and bacterial enzymatic which develops peculiar color which is species specific.



Biochemical tests

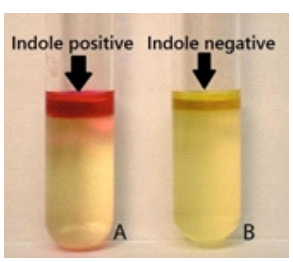
Oxidase Test

The oxidase test detects the presence of a cytochrome oxidase system that will catalyze the transport of electrons between electron donors in the bacteria and a redox dye- tetramethyl-p-phenylene-diamine, this test can be performed using Oxidase Disc (ODV, single vial, 50 Discs). The dye is reduced to deep purple color. This test is used to assist in the identification of *Pseudomonas, Neisseria, Alcaligens, Aeromonas, Moraxella, Campylobacter, Vibrio, Brucella* and *Pasteurella* species (oxidase positive), all of which produce the enzyme cytochrome oxidase. The oxidase test is used to determine if an organism possesses the cytochrome oxidase enzyme. All Enterobacteriaceae are oxidase negative except *Pleisomonas shigelloides*.

Most commonly practiced method is Dry filter paper method, since the oxidase reagent is unstable and has to be freshly prepared for use, this method is convenient. In this method, discs of Whatman's No. 1 filter paper impregnated with 1% solution of tertramethyl-p-phenylene-diamine dihydrochloride, are moistened with distilled water and the colony to be tested is picked up with a chromium loop and smeared over the moist area. Result Interpretation of Oxidase Test: Positive result is development of a deep purple-blue/blue colour which indicates oxidase production within 5-10 seconds. Negative result is no purple-blue colour/no colour change.

Dry Filter Paper Method

Indole Test



The indole test determines the ability of an organism to produce indole from the degradation of the amino acid tryptophan. Indole production is detected by Kovac's or Ehrlich's reagent which contains 4 (p)-dimethylamino benzaldehyde, this reacts with indole to produce a red coloured compound. Indole test is a commonly used biochemical test (e.g. in IMVIC test Kit-207900011). It is an aid in differentiation of the Enterobacteriaceae and other genera.

Two methods are in use:

a) Conventional tube method requiring overnight incubation, which identifies weak indole producing organisms.
b) Spot indole test, which detects rapid indole producing organisms.

Result Interpretation of Indole test: Positive result is pink colored ring after addition of appropriate reagent;

Negative result is no color change even after the addition of appropriate reagent. *Klebsiella, Enterobacter, Hafnia* and species give variable indole reactions but are usually negative.

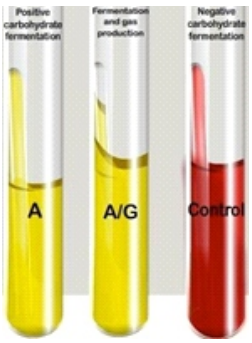
Most strains of *P. vulgaris, M. morganii, Providenica* and *Escherichia* species are positive for indole except *E. vulneris*.

In Diagnostic laboratories, Indole test can is performed using multi-test agar. Three most commonly used agar medium are:

Sulfide-indole-motility (SIM - AM50892) medium:

The SIM medium is a multi-test agar used to test for indole production while simultaneously determining motility and hydrogen sulfide producing abilities of the bacterial isolate.

Carbohydrates Fermentation Test



The carbohydrate fermentation test is used to determine whether or not bacteria can utilize a certain carbohydrate. It tests for the presence of acid and/or gas produced from the fermentation of a single particular carbohydrate. Carbohydrate fermentation patterns are useful in differentiating among bacterial groups or species. Lactose fermentation exhibits variable results depending on the genus and species.

All members of Enterobacteriaceae family are glucose fermenters (they can metabolize glucose anaerobically).

Maltose fermentation differentiates *Proteus vulgaris* (positive) from *Proteus mirabilis* (negative).

Both *Neisseria gonorrhoeae* (gonococci) and *Neisseria meningitides* (meningococci) ferments glucose, but only meningococci ferments maltose.

Rapid carbohydrate utilization test can be performed to identity *Corynebacterium diphtheriae* and other *Corynebacterium* species.

Basal medium containing a single carbohydrate source such as Glucose, Lactose, Sucrose or any other carbohydrate is used for this purpose. Phenol Red Carbohydrate Fermentation Broth media (AM508012, AM10802, AM10803, AM10805, AM10806, AM10807, AM10808) is most commonly used for this test. A pH indicator such as Andrade's solution, Bromocresol purple (BCP), Bromothymol blue (BTB) or Phenol red is also present in the medium; which will detect the lowering of the pH of the medium due to acid production. Small inverted tubes called Durham tube is also immersed in the medium to test for the production of the gas (hydrogen or carbon dioxide). If the test organism produces gas, the gas displaces the media present inside the tube and gets trapped producing a visible air bubble. Common end-products of bacterial fermentation include lactic acid, formic acid, acetic acid, butyric acid, butyl alcohol, acetone, ethyl alcohol, carbon dioxide and hydrogen.

Figure 1. Peptone media with phenol red indicator. From left to right: Uninoculated tube; glucose fermenter with gas production (visible air bubble in the inverted Durham tube); glucose fermenter without gas production (no visible air bubble in the inverted Durham tube); non-fermenter.

The production of the acid lowers the pH of the test medium, which is detected by the color change of the pH indicator. Color change only occurs when sufficient amount of acid is produced, as bacteria may utilize the peptone producing alkaline by products.

Based on the characteristics reactions observed, bacteria can be classified as: Fermenter with acid production only, fermenter with acid and gas production and non-fermenter.

We have compiled briefly the identification of clinically important Enterobacteriaceae and the principle as well as the technical information of the elementary identification. In next article, elementary identification of Enterobacteriaceae based on genomic level / molecular level techniques available will be briefed.

Waterless Scrub

Abstract
A new waterless surgical hand scrub product containing 1% chlorhexidine gluconate (CHG) and 61% ethyl alcohol in an emollient-rich lotion base (CHG/ethanol-emollient hand preparation) was evaluated. Clinical studies were based on the Tentative Final Monograph for Health Care Antiseptic Drug Products (TFM); Proposed Rule and ASTM E1115-91, Standard Test Method for Evaluation of Surgical Hand Scrub Formulations.

Two randomized, blinded well-controlled clinical studies involving over 100 healthy subjects evaluated the antimicrobial effectiveness of CHG/ethanol-emollient hand preparation in producing an immediate and persistent reduction in the normal bacterial flora of the hands. CHG/ethanol-emollient hand preparation was applied without scrubbing or the use of water, while a 4% CHG reference product was applied using scrub brushes in two traditional 3-minute surgical scrubs.

Over a 5-day period, each subject performed a series of 11 surgical scrubs using one of the products. After the first treatment on Days 1, 2 and 5, surgical gloves were worn for 3 and/or 6 hours. Bacterial samples were taken using the glove juice technique at 1 minute, 3 hours and/or 6 hours after treatment. The immediate bactericidal effect of CHG/ethanol-emollient hand preparation after a single application resulted in a 2.5 log reduction in normal flora. This bactericidal effect persisted throughout the study, and eventually increased to a 3.5 log reduction after the eleventh scrub on Day 5. The log reductions of CHG/ethanol-emollient hand preparation proved to be significantly better ($p<0.5$) than that of the 4% CHG product at each sampling interval on Days 1 and 2, and at the 6 hour sampling on Day 5, exceeding the TFM requirements. Use of this new waterless product as a surgical hand scrub lowers bacterial flora on the hands.

Introduction
This white paper describes the results of two clinical studies designed to determine the antimicrobial effectiveness of CHG/ethanol-emollient hand preparation using the log reduction criteria for bacterial counts on the hands defined by the Food & Drug Administration's (FDA) Tentative Final Monograph for Health-Care Antiseptic Drug Products (TFM). In these trials, CHG/ethanol-emollient hand preparation is compared with Hibiclens® (Stuart Pharmaceuticals, Wilmington, DE), a currently marketed presurgical antimicrobial hand-wash product containing 4% CHG in a detergent base. Changes in baseline skin condition were also measured based on results of subject self-assessment questionnaires.

Objectives
- To evaluate the effectiveness of the CHG/ethanol-emollient hand preparation formulation as a surgical hand scrub in meeting the TFM criteria for immediate and persistent reductions in the number of bacteria on the hands.
- To assess bacterial reductions achieved within 1 minute and at 3 and 6 hours post-treatment, comparing the CHG/ethanol-emollient hand preparation product versus Hibiclens.
- To compare the skin condition of the hands as assessed by subjects receiving the CHG/ethanol-emollient hand preparation product to that of subjects receiving Hibiclens.

Methods
Study design
Two prospective, randomized, partially-blinded, parallel-group trials (the design was identical for Studies A and B):
- 14-day pretreatment washout period for stabilization of hand bacterial flora, during which subjects refrained from using any topical antimicrobials, systemic antibiotics, or medicated soaps, lotions, shampoos, etc.
- 5 to 7 days of baseline bacterial evaluations where three baseline samples of hand bacterial flora were taken. Subjects with baseline bacterial populations ≥ 1.0 x 105 colony forming units (CFU) per hand at the first and second baseline samplings were eligible to be enrolled in the treatment period.
- 5-day treatment period during which subjects performed a series of 11 simulated surgical hand scrubs using one of the test products:
  - once daily on Treatment Days 1 and 5, and
  - three times daily on Treatment Days 2, 3, and 4.

Treatment
Subjects were randomized to receive one of the following two* treatments during each hand wash procedure:
- CHG/ethanol-emollient hand preparation (6 mL, 3 x 2 mL), or
- Hibiclens (10 mL, 2 x 5 mL).

* Note: In one of the two studies, some subjects were also randomized to receive a vehicle control formulation. Those data are not presented here.

Bacterial Samples
- Samples were collected following scrubs on Treatment Days 1, 2 and 5.
- Hands were randomized to bacterial sampling times. The first hand was sampled at 1 minute or 3 hours after scrubbing. The second hand of each subject was then sampled at either 3 or 6 hours after scrubbing.
- Sampling technique:
  - Loosely fitting sterile surgical gloves were placed over the hands to be sampled, then 75 mL of sampling solutions was aseptically added to the gloves.
  - Gloves were occluded above the wrist and the gloved hand was uniformly massaged for 1 minute.
  - After massaging, an aliquot of the fluid in the glove was aseptically transferred to a serial dilution tube containing suitable antimicrobial neutralizers to achieve a 1:10 dilution.
  - Solutions were plated using Trypticase Soy Agar and incubated for 48 to 72 hours at 30°C ± 2°C. Colonies were counted and viable cells in the undiluted sample were calculated by standard methods.
- Log reductions in bacterial counts were measured after 1 minute, 3 hours, and at 6 hours on Days 1, 2, and 5.
- Reductions in bacterial counts achieved with CHG/ethanolemollient hand preparation were compared with those of a reference control treatment (Hibiclens).

Subject

Healthy, male or female volunteer subjects, ages 18 to 65 years old, inclusive, with 1st and 2nd baseline counts ≥ 1.0 x 105 CFU per hand. Demographic and baseline characteristics of the study population were similar across test groups. (Table 1)

Table 1. Demographic characteristics

Parameter	Study A (HTR)		Study B (VML)	
	CHG/ethanol-emollient hand preparation (N=27)	Hibiclens (N=25)	CHG/ethanol-emollient hand preparation (N=27)	Hibiclens (N=25)
Age Years				
Mean (SD)	51.3 (10.3)	54.8 (7.8)	30.1 (7.3)	27.9 (7.5)
Gender N (%)				
Male	4 (15)	7 (28)	11 (32)	7 (35)
Female	23 (85)	18 (72)	23 (68)	13 (65)
Race N (%)				
White	27 (100)	22 (88)	31 (91)	20 (100)
Black	-	3 (12)	-	-
Hispanic	-	-	3 (9)	-

Evaluation Criteria

Efficacy:

Efficacy evaluations were based on the immediate and persistent activity of CHG/ethanol-emollient hand preparation as measured by the log reductions from baseline counts per hand at the following post-scrub sampling time points:

- Treatment Day 1 at 1 minute, 3 hours, and 6 hours.
- Treatment Day 2 (after the 1st scrub) at 1 minute, 3 hours, and 6 hours.
- Treatment Day 5 at 1 minute, 3 hours, and 6 hours.

Skin Condition:

Based on subject self-assessment questionnaires, change from baseline skin condition at Day 4 was calculated for several skin characteristics (appearance, intactness, moisture content, and sensation), based on a seven-point scale (1=abnormal, red, dry itchy, etc., to 7=normal).

Safety:

Assessments based on observed and reported adverse events.

Statistical Methods

Efficacy:

- Raw data on microbial counts from each baseline determination on each hand (CFU/hand) were converted to base 10 logarithms, then were averaged to determine each hand's baseline count.
- Raw data on microbial counts from each baseline determination on each hand (CFU/hand) were converted to base 10 logarithms, then were averaged to determine each hand's baseline count.
- The differences between groups in log reductions at each time period were analyzed using a t-test, with significance at **p greater than or equal to 0.05 (2-tailed)**.

Skin Condition:

- Change from baseline at Day 4 was calculated for each item on the subject self-assessment questionnaire.
- A one-way analysis of variance (ANOVA) on the rank-transformedchange scores was used to test the effect of the formulation on each aspect of skin condition.

Results

Disposition of subjects is displayed in Table 2.

Category	Study A		Study B	
	CHG/ethanol-emollient hand preparation	Hibiclens	CHG/ethanol-emollient hand preparation	Hibiclens
Enrolled	27	25	34	20
CompletedStudy	24	24	31	19
Reasons for Discontinuation* Adverse Event	2	0	1	0
Personal reasons	2	1	-	-
Lack of compliance	-	-	2	1
Lost to follow-up	1	0	-	-

*More than one reason for discontinuing could be provide.

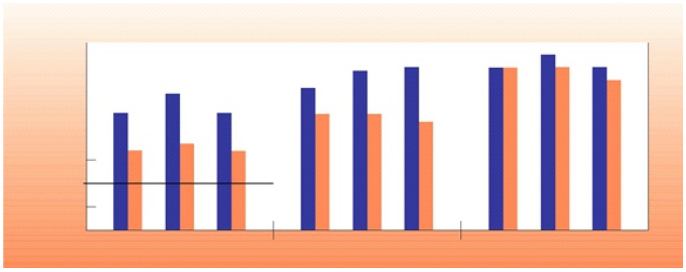
In Study A, both the CHG/ethanol-emollient hand preparation and Hibiclens groups showed statistically significant reductions from baseline bacterial counts at all time points. The log reductions from baseline bacterial counts on Days 1, 2, and 5 exceeded the TFM criteria at the specified time points for both groups (Table 3). In comparing CHG/ethanol-emollient hand preparation and Hibiclens, CHG/ethanol-emollient hand preparation had significantly greater log reduction at 1 minute and 3 hours on Day 1 and6 hours on Day 2. In Study B, the log reductions from baseline bacterial counts were statistically significant and exceeded the TFM criteria at the specified time points for both CHG/ethanol-emollient hand preparation and Hibiclens. In comparing CHG/ethanol-emollient hand preparation and Hibiclens, CHG/ethanol-emollient hand preparation had statistically significantly greater log reductions in bacteria at 3 and 6 hours on Day 1 and at all time points on Day 2 (Table 3).

Table 3: Log reductions in bacterial counts (CFU/Hand) from baseline

	Study A		Study B	
	CHG/ethanol-emollient hand preparation	Hibiclens	CHG/ethanol-emollient hand preparation	Hibiclens
Baseline Period Period Mean	6.3	6.4	6.1	6.0
Day 1 Log Reduction				
1 Minute	2.5*	1.8	2.5	1.6
3 Hours	2.6*	1.8	3.1*	1.8
6 Hours	2.2	1.9	2.8*	1.4
Day 2 Log Reduction				
1 Minute	3.0	2.6	3.2*	2.4
3 Hours	3.1	2.7	3.7*	2.3
6 Hours	3.3*	2.3	3.6*	2.3
Day 5 Log Reduction				
1 Minute	3.7	3.7	3.5	3.6
3 Hours	3.6	3.7	3.9	3.6
6 Hours	3.8	3.5	3.5	3.0

*Statistically significantly higher for CHG/ethanol-emollient hand preparation than for Hibiclens.

Figure 1. Combined Analysis



*Statistically significant difference.

When data from the two studies were combined, CHG/ethanol-emollient hand preparation had statistically significantly greater log reductions in bacteria at all time points on Days 1 and 2 and at the 6-hour sampling on Day 5 compared to Hibiclens (Figure 1).

Skin Assessments

In Study A, at the end of Day 4, CHG/ethanol-emollient hand preparation was statistically significantly superior to Hibiclens with respect to change from baseline moisture content ($p=0.0091$), although no statistically significant differences were found for appearance, intactness, or sensation.

In Study B, a statistically significant treatment effect was demonstrated for all skin assessments, indicating that CHG/ethanol-emollient hand preparation was associated with better skin condition than Hibiclens. Pair wise comparisons of CHG/ethanol-emollient hand preparation and Hibiclens yielded statistically significant results for all skin condition assessments (appearance, intactness, moisture content, and sensation) in favour of CHG/ethanol-emollient hand preparation.

Safety

No serious or severe adverse events occurred during either study. Two subjects reported three adverse events in the CHG/ethanol-emollient hand preparation groups, which were "probably related" to the study formulation:

- One subject reported a maculopapular rash on the dorsal surface of both wrists where the gloves had been secured.
- One subject experienced two adverse events— conjunctivitis and abnormal vision—after rubbing his eyes after application.

Four other reported adverse events which were "probably not related" to study formulation included: a viral infection, menorrhagia, an upper respiratory infection, and an inflicted injury of cuts to the knuckles of one hand.

Two adverse events were reported with the use of Hibiclens:

- One subject experienced an allergic reaction considered "possibly related" to use of the product.
- One subject experienced an erythematous rash considered "probably not related" to use of the product.

Conclusions

- CHG/ethanol-emollient hand preparation met or exceeded TFM criteria for antimicrobial effectiveness.
- CHG/ethanol-emollient hand preparation was equal or superior to Hibiclens in antimicrobial effectiveness, as assessed by log reductions in counts of hand bacteria.
- CHG/ethanol-emollient hand preparation was associated with less drying of the skin than Hibiclens, as assessed by subject evaluations of Moisture Content at the end of Day 4 in Study A, and with statistically significantly better skin condition scores for appearance, intactness, moisture content, and sensation scores than Hibiclens in Study B.
- CHG/ethanol-emollient hand preparation was well tolerated in both studies.

References

1. Federal Register Part III, Tentative Final Monograph for Health-Care Antiseptic Drug Products; Proposed Rule. Vol. 59, No 116 (Friday, June 17, 1994). Code of Federal Regulations, Title 21 CFR Parts 333 and 369.

2. ASTM Standard 1115-91. Standard Test Method for Evaluation of Surgical Hand Scrub Formulations. Annual Book of ASTM Standards, Vol. 11.05., p. 447-450, 1996.

Venkatraman Ramakrishnan

Venkatraman "Venki" Ramakrishnan was born in 1952 is an American and British structural biologist of Indian origin. He was elected President of the Royal Society in November 2015; Presidents serve for five years. In 2009 he shared the Nobel Prize in Chemistry with Thomas A. Steitz and Ada Yonath, "for studies of the structure and function of the ribosome. Since 1999, he has worked as a group leader at the Medical Research Council (MRC) Laboratory of Molecular Biology (LMB) on the Cambridge Biomedical Campus, UK.

EDUCATION & EARLY LIFE

Ramakrishnan was born in Chidambaram in Cuddalore district of Tamil Nadu, India to C. V. Ramakrishnan and Rajalakshmi Ramakrishnan in a Tamil Iyer family. Both his parents were scientists, and his father was head of the Department of Biochemistry at the Maharaja Sayajirao University of Baroda. At the time of his birth, Ramakrishnan's father was away from India doing postdoctoral research with David E. Green at the —University of Wisconsin Madison in the US.

His mother obtained a PhD in Psychology from McGill University in 1959 which she completed in only 18 months, and was mentored by Donald O. Hebb. Lalita Ramakrishnan, his younger sister, is professor of immunology and infectious diseases at the Department of Medicine, University of Cambridge, and is a member of the National Academy of Sciences.

Ramakrishnan moved to Vadodara (previously also known as Baroda) in Gujarat at the age of three, where he had his schooling at Convent of Jesus and Mary, except for spending 1960–61 in Adelaide, Australia. Following his pre-science at the Maharaja Sayajirao University of Baroda, he did his undergraduate studies in the same university on a National Science Talent Scholarship, graduating with a Bachelor of Science degree in Physics in 1971.

At the time, the physics course at Baroda was new, and based in part on The Berkeley Physics Course and The Feynman Lectures on Physics.

Immediately after graduation he moved to the U.S., where he obtained his PhD degree in Physics from Ohio University in 1976 for research into the ferroelectric phase transition of potassium dihydrogen phosphate (KDP) supervised by Tomoyasu Tanaka. He then spent two years studying biology as a graduate student at the University of California, San Diego while making a transition from theoretical physics to biology.

CAREER AND RESEARCH

Ramakrishnan began work on ribosomes as a postdoctoral fellow with Peter Moore at Yale University. After his post-doctoral fellowship, he initially could not find a faculty position even though he had applied to about 50 universities in the U.S.

He continued to work on ribosomes from 1983-95 as a staff scientist at Brookhaven National Laboratory In 1995 he moved to the University of Utah as a Professor of Biochemistry, and in 1999, he moved to his current position at the Medical Research Council Laboratory of Molecular Biology in Cambridge, England, where he had also been a sabbatical visitor during 1991-92.

In 1999, Ramakrishnan's laboratory published a 5.5 Angstrom resolution structure of the 30S subunit. The following year, his laboratory determined the complete molecular structure of the 30S subunit of the ribosome and its complexes with several antibiotics. This was followed by studies that provided structural insights into the mechanism that ensures the fidelity of protein biosynthesis. In 2007, his laboratory determined the atomic structure of the whole ribosome in complex with its tRNA and mRNA ligands. Since 2013, he has primarily used cryo-EM to determine new ribosome structures. Ramakrishnan is also known for his past work on histone and chromatin structure.

As of 2015 his most cited papers (according to Scopus) have been published in Nature, Science, and Cell. In an interview in July 2018, he said that Britain's decision to leave the European Union (Brexit) was hurting Britain's reputation as a good place to work in science, commenting "It's very hard for the science community to see any advantages in Brexit. They are pretty blunt about that." He saw advantages to both the UK and the EU for Britain to continue to be engaged in Galileo and Euratom, which, unlike the European Medicines Agency, are not EU agencies.

AWARDS AND HONOURS

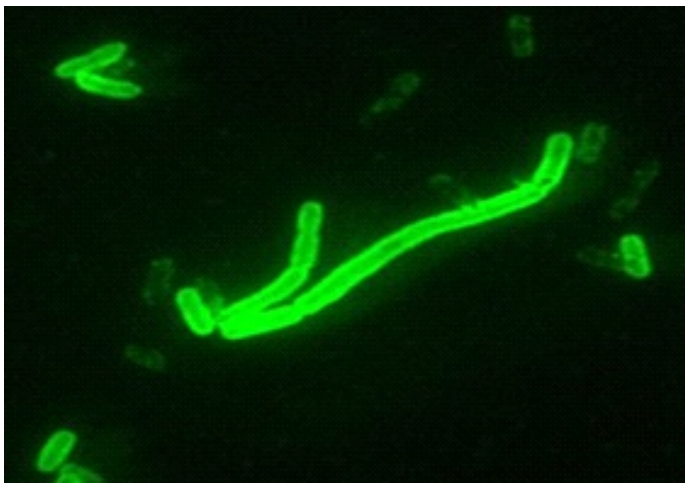
Ramakrishnan was elected a Member of the European Molecular Biology Organization (EMBO) in 2002, a Fellow of the Royal Society (FRS) in 2003, and a Member of the U.S. National Academy of Sciences in 2004. In 2007, Ramakrishnan was awarded the Louis-Jeantet Prize for Medicine and the Datta Lectureship and Medal of the Federation of European Biochemical Societies (FEBS). In 2008, he won the Heatley Medal of the British Biochemical Society. Since 2008, he is a Fellow of Trinity College, Cambridge and a foreign Fellow of the Indian National Science Academy. He was elected an Honorary Fellow of the Academy of Medical Sciences in 2010, and has received honorary degrees from the Maharaja Sayajirao University of Baroda, University of Utah and University of Cambridge. He is also an Honorary Fellow of Somerville College, Oxford.

In 2009, Ramakrishnan was awarded the Nobel Prize in Chemistry along with Thomas A. Steitz and Ada Yonath.He received India's second highest civilian honor, the Padma Vibhushan, in 2010. Ramakrishnan was knighted in the 2012 New Year Honours for services to Molecular Biology, but does not generally use the title 'Sir'. In the same year, he was awarded the Sir Hans Krebs Medal by the FEBS. In 2014, he was awarded the XLVI Jiménez-Díaz Prize by the Fundación Conchita Rábago (Spain). Ramakrishnan was included as one of 25 Greatest Global

Living Indians by NDTV Channel, India on 14 December 2013. His certificate of election to the Royal Society reads: Ramakrishnan is internationally recognised for determination of the atomic structure of the 30S ribosomal subunit. Earlier he mapped the arrangement of proteins in the 30S subunit by neutron diffraction and solved X-ray structures of individual components and their RNA complexes. Fundamental insights came from his crystallographic studies of the complete 30S subunit. The atomic model included over 1500 bases of RNA and 20 associated proteins. The RNA interactions representing the P-site tRNA and the mRNA binding site were identified and the likely modes of action of many clinically important antibiotics determined. His most recent work goes to the heart of the decoding mechanism showing the 30S subunit complexed with poly-U mRNA and the stem-loop of the cognate phenylalanine tRNA. Anti-codon recognition leaves the "wobble" base free to accommodate certain non-Watson/Crick basepairs, thus providing an atomic description of both codon:anti-codon recognition and "wobble". He has also made substantial contributions to understanding how chromatin is organised, particularly the structure of linker histones and their role in higher order folding.

1) "The Way To Get Started Is To Quit Talking And Begin Doing." – Walt Disney
2) "The Pessimist Sees Difficulty In Every Opportunity. The Optimist Sees Opportunity In Every Difficulty." – Winston Churchill
3) Don't Let Yesterday Take Up Too Much Of Today." – Will Rogers
4) "You Are Never Too Old To Set Another Goal Or To Dream A New Dream." – C.S. Lewis
5) It's Not Whether You Get Knocked Down, It's Whether You Get Up." –Vince Lombardi
6) If You Are Working On Something That You Really Care About, You Don't Have To Be Pushed. The Vision Pulls You." – Steve Jobs
7) "People Who Are Crazy Enough To Think They Can Change The World, Are The Ones Who Do." – Rob Siltanen
8) "Failure Will Never Overtake Me If My Determination To Succeed Is Strong Enough." – Og Mandino
9) "Entrepreneurs Are Great At Dealing With Uncertainty And Also Very Good At Minimizing Risk. That's The Classic Entrepreneur." – Mohnish Pabrai
10) "We May Encounter Many Defeats But We Must Not Be Defeated." – Maya Angelou
11) "Knowing Is Not Enough; We Must Apply. Wishing Is Not Enough; We Must Do." – Johann Wolfgang Von Goethe
12) "Imagine Your Life Is Perfect In Every Respect; What Would It Look Like?" – Brian Tracy
13) "We Generate Fears While We Sit. We Overcome Them By Action." – Dr. Henry Link
14) "Whether You Think You Can Or Think You Can't, You're Right." – Henry Ford
15) "Security Is Mostly A Superstition. Life Is Either A Daring Adventure Or Nothing." – Helen Keller

Yersinia Pestis



Higher order taxa
Kingdom: Eubacteria
Phylum: Proteobacteria
Class: Gamma Proteobacteria
Order: Enterobacteriale
Genus: Yersinia

DESCRIPTION & SIGNIFICANCE

Yersinia pestis was discovered in Hong Kong in 1894 by a Swiss physician Alexandre Yersin, who was a student of the Pasteur school of thought. He linked *Y. pestis* to the bubonic plauge, an epidemic that ravaged Europe during the 1300s. The organism was isolated during a outbreak in Hong Kong, a new geographical region for the organism that has been seen in Europe and Africa.
It is very important to have the genome sequenced for *Y. pestis* because this organism is capable of causing very fatal diseases. Since scientists were able to sequence the genome, they now have information of how diseases caused by this pathogenic bacteria develop and also the evolutionary history of the bacteria. Having the genome sequence also means that they are able to determine other species that are related to *Yersinia pestis* which can prevent future outbreaks.

CELL STRUCTURE AND METABOLISM

Yersinia pestis is a rod shaped gram-negative bacteria that can also have a spherical shape. It is also covered by a slime envelope that is heat labile. When the bacteria is in a host, it is nonmotile (incapable of self-propelled movement), but when isolated it is motile.
Y. pestis uses aerobic respiration and anaerobic fermentation to produce and consume hydrogen gas for energy.

ECOLOGY

Yersinia pestis interacts mainly with rodents such as rats and fleas. Through these carriers, *Yersinia pestis* is able to invade human cells and create diseases. *Yersinia pestis* are not rich in nuterients and can grow at temperatures ranging from about 26 Celcius to 37 Celcius.

PATHOLOGY

Y. pestis causes diseases through the bite of an infected rat or flea, but can also be transmitted by air. Fleas can become infected by taking the blood of other infected animals. *Y. pestis* grows in the midgut and eventually blocks the proventriculus, starving the flea for blood. The insects attempt to feed more often but end up giving back infected blood into the wound of the bite.

Symptoms include:

- Sudden onset of high fever
- Emergence of a smooth, painful swelling of the lymph gland, called a buboe. The most common area is the groin, but swollen glands may also occur in the armpits or neck. Pain may occur in the area before the swelling.
- Chills
- General discomfort or ill feeling (malaise)
- Muscular pain
- Severe headache
- Seizures

The major defense against *Y pestis* infection is the development of specific anti-envelope antibodies, which serve as opsonins for the virulent organisms, allowing their rapid phagocytosis and destruction while still within the initial infectious locus. The immune mechanism against this disease is extremely complex and involves a combination of humoral and cellular factors. The host is immune to virulent rechallenge, the inoculum being eliminated as though the organisms were completely avirulent". Killed *Y. pestis* vaccines induce some measure of host protection, although it is less effective.
Yersinia pestis infections must be diagnosed quickly due to the high virulence of these organisms. Death from pneumonic plague can occur in as little as 24 hours after the first appearance of symptoms.
Yersinia pestis can be killed with mild heat (55°C) and by treatment with 0.5 percent phenol for 15 minutes. It is susceptible to sulfadiazine, streptomycin, tetracycline, and chloramphenicol.

CURRENT RESEARCH

Researchers recently compared the strains of *Yersinia pestis* (strain C092 and KIM) and *Yersinia pseudotuberculosis*. This comparison would show how the pathogen could have evolved from each other within a few hundred years by acquiring new genes. These new genes can also inactivate some of the existing ones. In the end, Jargid et al determined that both strains could have diverged from a common ancestor thousands of years ago, however the lack of a reliable molecular clock, IS transposition and gene inactivation made it difficult to specifically determine the actual distance between both species. They also concluded that gene inactivation could have lead to the different genes.
Another research showed a recent emergence of new variants of *Yersinia pestis* in Madagascar. The researchers reported that the *Y. pestis* strain in Madagascar before 1982 was of the ribotype B, but after 1982 strains with ribotypes R, Q and T were isolated on a high plateau on the island .The researchers concluded that none of the strains studied from anywhere else in the world displayed

these new ribotypes, that the new variants were not isolated on the seaport but on the high plateau and that the new strains were discovered after a recent plague surveillance was established.

Researchers have also detected the *Yersinia pestis* organism on a 400 year old set of teeth. They performed this research because the lack of suitable infected material has prevented direct discovery of the plague, thus making the idea of a black plague hypothetical. "The durability of dental pulp, together with its natural sterility, makes it a suitable material on which to conduct research". They used bodies of people who died from the plague in Europe. "PCRs incorporating ancient DNA extracts and primers specific for the human beta-globin gene demonstrated the absence of inhibitors in these preparations. The incorporation of primers specific for *Y. pestis* rpoB and the recognized virulence-associated pla repeatedly yielded products that had a nucleotide sequence indistinguishable from that of modern day isolates of the bacterium". The researchers were able to confirm that the disease was present at the end of the 16th century due to a nucleic acid-base confirmation. Many gram-negative bacteria (including *E. tarda*) use ferric uptake regulator (Fur) as an important transcriptional regulator. In *E. tarda*, Fur's role is significant and multifaceted; Fur affects the growth, siderophore production, and acid tolerance of *E. tarda*, helps protect against oxidative stress and host serum, helps inhibit host immune response, and generally increases the overall virulence of the bacterium. *Y. pestis* is well known as a flea-borne bacteria. However, it evolved from *Y. pseudotuberculosis*, which exhibited significant oral toxicity to flea vectors. This oral toxicity is caused by urease activity which has been silenced in *Y. pestis*, due to expression of the mutant ureD allele. Restoration of functional ureD rendered *Y. pestis* toxic to vectors, which may be invaluable knowledge for decreasing plague transmission.

Bioreactor Device helps Frogs regenerate their limbs

A team of scientists designed a device that can induce partial hindlimb regeneration in adult aquatic African clawed frogs (*Xenopuslaevis*) by "kick-starting" tissue repair at the amputation site. Their findings, appearing November 6 in the journal Cell Reports, introduce a new model for testing "electroceuticals," or cell-stimulating therapies.

"At best, adult frogs normally grow back only a featureless, thin, cartilaginous spike," says senior author Michael Levin, developmental biologist at the Allen Discovery Center at Tufts University. "Our procedure induced a regenerative response they normally never have, which resulted in bigger, more structured appendages. The bioreactor device triggered very complex downstream outcomes that bioengineers cannot yet micromanage directly."

The scientists 3-D printed the bioreactor out of silicon and filled it with hydrogel—a sticky glob of polymers. They laced the hydrogel with hydrating silk proteins that promote healing and regeneration, then added progesterone. Progesterone is best known for its role in preparing the uterus for pregnancy, but the hormone has also been shown to promote nerve, blood vessel, and bone tissue repair.

The researchers split the frogs into three groups: experimental, control, and sham. For the experimental and sham group, they sutured the device on the frogs immediately after limb amputation. In the experimental group, the bioreactor released progesterone onto the amputation site. In all cases, they removed the devices after 24 hours.

When they looked at the experimental group frogs at different time points over 9.5 months, they noticed that the bioreactor seemed to trigger a degree of limb regeneration not observed in the other groups. Instead of a typical spike-like structure, the bioreactor treatment resulted in a paddle-like formation closer to a fully formed limb than unaided regeneration could create.

"The bioreactor device created a supportive environment for the wound where the tissue could grow as it did during embryogenesis," says Levin. "A very brief application of bioreactor and its payload triggered months of tissue growth and patterning."

Xenopuslaevis swimming in a tank pre-amputation.

Levin and his team took a closer look at the regenerated structures using molecular and histology analyses. They saw that, unlike in the control and sham groups, the regenerating limbs of the bioreactor-treated frogs were thicker with more developed bones, innervation, and vascularization.

Analyzing video footage of the frogs in their tanks, they also noticed that the frogs could swim more like unamputated frogs.

RNA sequencing and transcriptome analysis revealed that the bioreactor had altered the gene expression occurring in cells at the amputation site. Genes involved in oxidative stress, serotonergic signalling, and white blood cell activity were upregulated, while some other signaling-related genes were downregulated.

The researchers also observed that scarring and immune responses were downregulated in the bioreactor-treated frogs, suggesting that the added progesterone dampened the body's natural reaction to injury in a way that benefited the regeneration process.

"In both reproduction and its newly discovered role in brain functioning, progesterone's actions are local or tissue-specific," says first author Celia Herrera-Rincon, neuroscientist in Levin's lab at Tufts University. "What we are demonstrating with this approach is that maybe reproduction, brain processing, and regeneration are closer than we think. Maybe they share pathways and elements of a common—and so far, not completely understood—bioelectrical code."

Levin's lab will continue to target bioelectric processes for inducing spinal cord regeneration and tumor reprogramming. They also hope to replicate their bioreactor experiment in mammals. Previous research suggests that mice can partially regenerate amputated fingertips in the right conditions, but their life on land hinders this process.

"Almost all good regenerators are aquatic," says Levin. "You can imagine why this matters: a mouse that loses a finger or hand, and then grinds the delicate regenerative cells into the flooring material as it walks around, is unlikely to experience significant limb regeneration."

Levin plans to next add sensors to the device for remote monitoring and optogenetic stimulation, which he hopes will improve control over cellular decision making after injury.

Best practices to manage burn patients-2

The burns patient has the same priorities as all other trauma patients.

Assess:
- Airway
- Breathing: beware of inhalation and rapid airway compromise
- Circulation: fluid replacement
- Disability: compartment syndrome
- Exposure: percentage area of burn.

Essential management points:
- Stop the burning
- ABCDE (A—Airway, B—Breathing, C—Circulation, D—Neurological disability, E—Exposure with environmental control)
- Determine the percentage area of burn (Rule of 9's)
- Good IV access and early fluid replacement.

The severity of the burn is determined by:
- Burned surface area
- Depth of burn
- Other considerations.

Morbidity and mortality rises with increasing **burned surface area**. It also rises with increasing age so that even small burns may be fatal in elderly people.

Burn Management in Adults

The "Rule of 9's" is commonly used to estimate the burned surface area in adults.

- The body is divided into anatomical regions that represent 9% (or multiples of 9%) of the total body surface (Figure 7). The outstretched palm and fingers approximates to 1% of the body surface area.
- If the burned area is small, assess how many times your hand covers the area.
- Morbidity and mortality rises with increasing burned surface area. It also rises with increasing age so that even small burns may be fatal in elderly people.

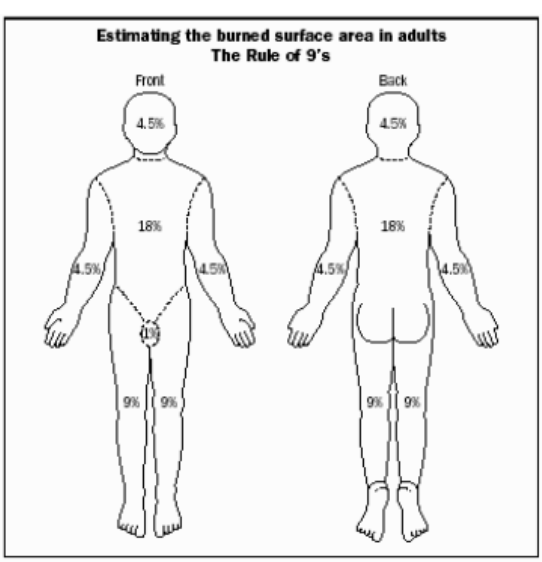
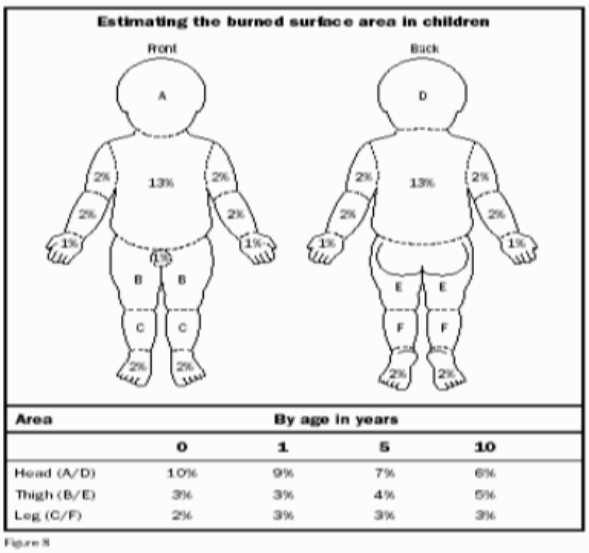


Figure 7

Burn Management in Children

- The 'Rule of 9's' method is too imprecise for estimating the burned surface area in children because the infant or young child's head and lower extremities represent different proportions of surface area than in an adult (see Figure 8).
- Burns greater than 15% in an adult, greater than 10% in a child, or any burn occurring in the very young or elderly are serious.



Area	By age in years			
	0	1	5	10
Head (A/D)	10%	9%	7%	6%
Thigh (B/E)	3%	3%	4%	5%
Leg (C/F)	2%	3%	3%	3%

Figure 8

Depth of Burn

It is important to estimate the depth of the burn to assess its severity and to plan future wound care. Burns can be divided into three types, as shown below.

Depth of Burn	Characteristics	Cause
First degree burn	• Erythema • Pain • Absence of blisters	• Sunburn
Second degree (Partial thickness)	• Red or mottled • Flash burns	• Contact with hot liquids
Third degree (Full Thickness)	• Dark and leathery • Dry	• Fire • Electricity or lightning • Prolonged exposure to hot liquids/ objects

It is common to find all three types within the same burn wound and the depth may change with time, especially if infection occurs. Any full thickness burn is considered serious.

Serious burn requiring hospitalization
- Greater than 15% burns in an adult
- Greater than 10% burns in a child
- Any burn in the very young, the elderly or the infirm
- Any full thickness burn
- Burns of special regions: face, hands, feet, perineum

- Circumferential burns
- Inhalation injury
- Associated trauma or significant pre-burn illness: e.g. diabetes

WOUND CARE

First Aid

- If the patient arrives at the health facility without first aid having been given, drench the burn thoroughly with cool water to prevent further damage and remove all burned clothing.
- If the burn area is limited, immerse the site in cold water for 30 minutes to reduce pain and edema and to minimize tissue damage.
- If the area of the burn is large, after it has been doused with cool water, apply clean wraps about the burned area (or the whole patient) to prevent systemic heat loss and hypothermia.
- Hypothermia is a particular risk in young children.
- First 6 hours following injury are critical; transport the patient with severe burns to a hospital as soon as possible.

Initial Treatment

- Initially, burns are sterile. Focus the treatment on speedy healing and prevention of infection.
- In all cases, administer tetanus prophylaxis.
- Except in very small burns, debride all bullae. Excise adherent necrotic (dead) tissue initially and debride all necrotic tissue over the first several days.
- After debridement, gently cleanse the burn with 0.25% (2.5 g/litre) chlorhexidine solution, 0.1% (1 g/litre) cetrimide solution, or another mild water-based antiseptic.
- Do not use alcohol-based solutions.
- Gentle scrubbing will remove the loose necrotic tissue. Apply a thin layer of antibiotic cream (silver sulfadiazine).
- Dress the burn with petroleum gauze and dry gauze thick enough to prevent seepage to the outer layers.

Daily Treatment

- Change the dressing daily (twice daily if possible) or as often as necessary to prevent seepage through the dressing. On each dressing change, remove any loose tissue.
- Inspect the wounds for discoloration or haemorrhage, which indicate developing infection.
- Fever is not a useful sign as it may persist until the burn wound is closed.
- Cellulitis in the surrounding tissue is a better indicator of infection.
- Give systemic antibiotics in cases of haemolytic streptococcal wound infection or septicaemia.
- Pseudomonas aeruginosa infection often results in septicaemia and death. Treat with systemic aminoglycosides.
- Administer topical antibiotic chemotherapy daily. Silver nitrate (0.5% aqueous) is the cheapest, is applied with occlusive dressings but does not penetrate eschar. It depletes electrolytes and stains the local environment.
- Use silver sulfadiazine (1% miscible ointment) with a single layer dressing. It has limited eschar penetration and may cause neutropenia.
- Mafenide acetate (11% in a miscible ointment) is used without dressings. It penetrates eschar but causes acidosis. Alternating these agents is an appropriate strategy.
- Treat burned hands with special care to preserve function.
  - Cover the hands with silver sulfadiazine and place them in loose polythene gloves or bags secured at the wrist with a crepe bandage;
  - Elevate the hands for the first 48 hours, and then start hand exercises;
  - At least once a day, remove the gloves, bathe the hands, inspect the burn and then reapply silver sulfadiazine and the gloves;
  - If skin grafting is necessary, consider treatment by a specialist after healthy granulation tissue appears.

Healing Phase

- The depth of the burn and the surface involved influence the duration of the healing phase. Without infection, superficial burns heal rapidly.
- Apply split thickness skin grafts to full-thickness burns after wound excision or the appearance of healthy granulation tissue.
- Plan to provide long-term care to the patient.
- Burn scars undergo maturation, at first being red, raised and uncomfortable. They frequently become hypertrophic and form keloids. They flatten, soften and fade with time, but the process is unpredictable and can take up to two years.
- In children
  - The scars cannot expand to keep pace with the growth of the child and may lead to contractures.
  - Arrange for early surgical release of contractures before they interfere with growth.
- Burn scars on the face lead to cosmetic deformity, ectropion and contractures about the lips. Ectropion can lead to exposure keratitis and blindness and lip deformity restricts eating and mouth care.
- Consider specialized care for these patients as skin grafting is often not sufficient to correct facial deformity.

Nutrition

- Patient's energy and protein requirements will be extremely high due to the catabolism of trauma, heat loss, infection and demands of tissue regeneration. If necessary, feed the patient through a nasogastric tube to ensure an adequate energy intake (up to 6000 kcal a day).
- Anaemia and malnutrition prevent burn wound healing and result in failure of skin grafts. Eggs and peanut oil and locally available supplements are good.

References:

https://www.who.int/surgery/publications/Burns_management.pdf

Highlights of the coming issue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