Epigenetics in the Modern Era: Taming the Wolf at the Door?

2011 NASA Occupational Health Meeting

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DISCLOSURES

Speaker's Bureau with the following entities: Care Fusion, Cubist, Glaxo-SmithKline, Pfizer, Ortho-McNeil, Merck

No mention of off-label usage will be employed in this program

Objectives

1. **Understand the status and limitation of the antibiotic armamentarium in 2011.**
2. **Understand the emergence of Epigenetics as it relates to oncogenesis and opportunities for counter-strategies in preventive medicine.**
3. **Understand novel delivery systems for vaccine therapy and disease prevention including Disaster Planning.**
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MICROBIAL MATH

3.5 billion years: microbes on planet earth

- 0.5 billion years: mammals on planet earth

3.0 billion years evolutionary head start

. . . Implications

HUMAN: BACTERIAL INTERFACE

- Total human cells/person ~ 10^{13}
- Total colonizing microbes ~ 10^{14}

. . . We are outnumbered 10:1!

NEJM 2010;362:75

MACRO TRENDS

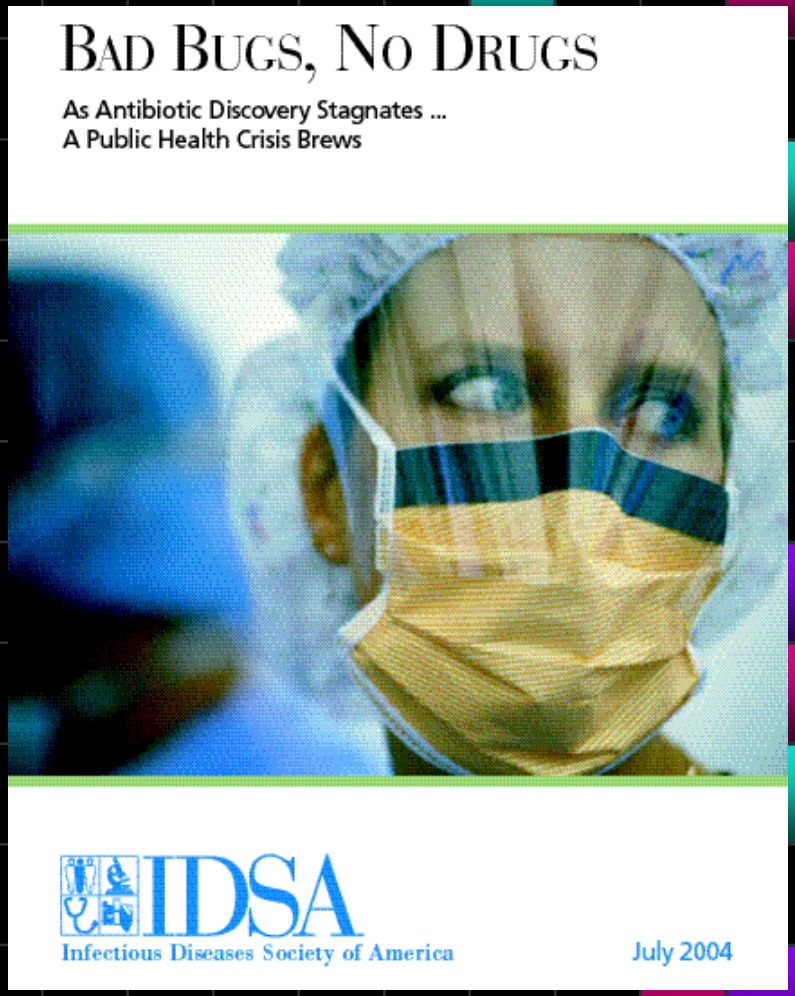
	1900	2025
% Population > 65 yrs	4% (3M)	22% (69M)

US Bureau of Vital Statistics

BAD BUGS, NO DRUGS[1]

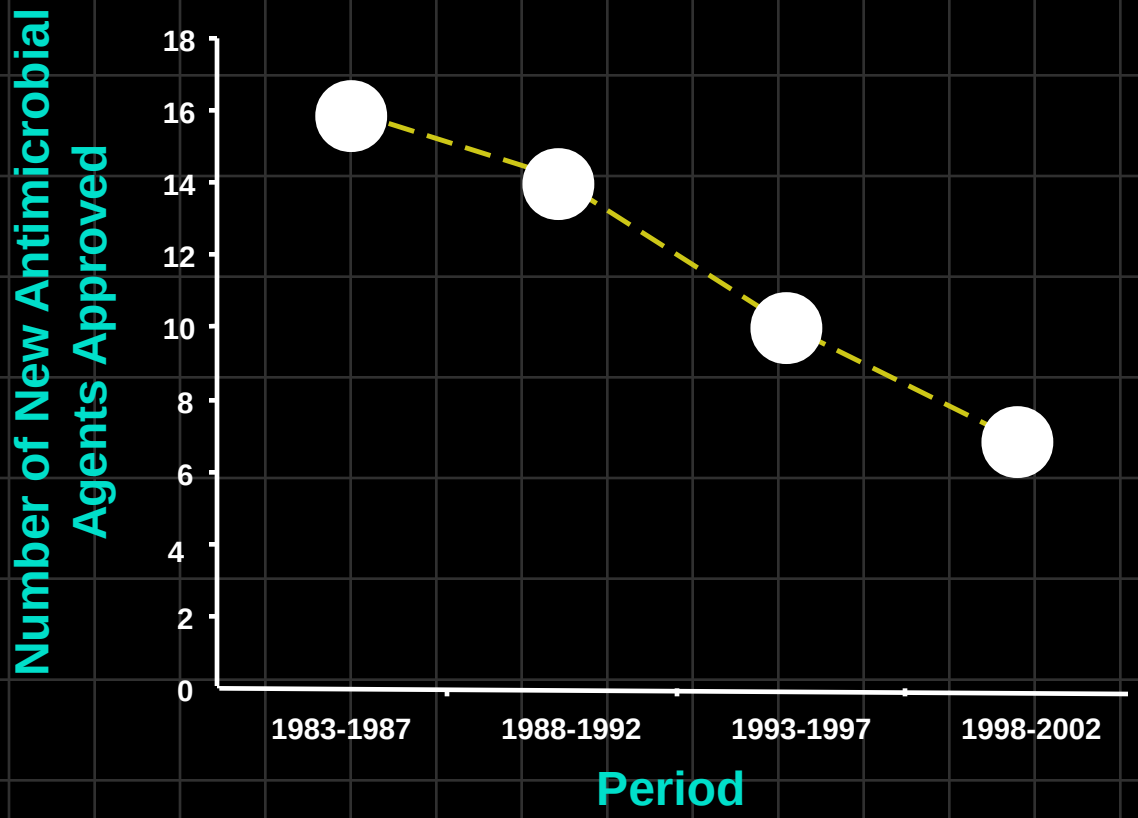
- Declining research investments in antimicrobial development[2]

- The Antimicrobial Availability Task Force of the IDSA identified problematic pathogens including gram-negative bacteria[2]



1. Infectious Diseases Society of America. Bad bugs, no drugs: as antibiotic discovery stagnates, a public health crisis brews. July, 2004. http://www.idsociety.org/WorkArea/showcontent.aspx?id=5554. Accessed October 30, 2007. 2. Talbot GH, et al. *Clin Infect Dis*. 2006;42:657-68.

DECLINING NUMBER OF ANTIMICROBIAL AGENTS APPROVED SINCE 1983



Adapted with permission from Spellberg B, et al. *Clin Infect Dis*. 2004;38:1279-1286.

- **Of 89 drugs approved in 2002, none was an antimicrobial[1]**

1. Infectious Diseases Society of America. Bad bugs, no drugs: as antibiotic discovery stagnates, a public health crisis brews. July, 2004. http://www.idsociety.org/WorkArea/showcontent.aspx?id=5554. Accessed October 30, 2007. 2. Spellberg B, et al. *Clin Infect Dis*. 2004;38:1279-1286.

"ESKAPE" Pathogens[1]

Clinical Outcomes:Mortality

	VRE	VSE	
Bacteremia[2]	n=683	n=931	OR, 2.52*
	MRSA	MSSA	
Bacteremia[3]	11,8% (n=382)	5,1% (n=433)	p<.001
	KPN-ESBL+	KPN-ESBL-	
Bacteremia[4]	52% (n=48)	31% (n=99)	p<0.05
	AB (IMP-R)	AB (IMP-S)	
Bacteremia[5]	57,5% (n=40)	27,5% (n=40)	p=0.007
	MDR-Pae	No-MDR-Pae	
Bacteremia[6]	21% (n=40)	12% (n=40)	p=0.08
	EB (IMP-R)	EB (IMP-S)	
Serious infections[7]	33% (n=33)	9% (n=33)	p=0.038

ESKAPE Reference:

J Infect Dis 2008; 41: 327

Bad Bugs Need Drugs

10x'20

Ten new **ANTIBIOTICS** by 2020

CID 2010;50:1081

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EPIGENETICS: HISTORY

- 1911: Rous reported that cell-free filtrates from birds with sarcoma could cause sarcoma in healthy birds

  ∴ nexus of microbiology and cancer

- 1966: Rous received Nobel Prize in physiology in medicine

J Exp Med 1911;13:397

EPIGENETICS: WHY AND HOW

- Why does human infection sometimes remain controlled vs progressing to malignant tumor at other times?

- Answer: Epigenetic modifications

JAMA 2011;305:1484

EPIGENETICS: MODERN ERA

- More than 20% of cancers causally linked to human pathogens

Virology 2009;392:1

ONCOGENIC BACTERIA: H. PYLORI

- Infects ~ 50% world's population
- WHO designates as class 1 carcinogen

- Linked to 65% gastric cancer in 1991

  Am J Gastroenterol 2005:100:210

  *Methylation of gastric cell promoter genes ↑'d 5-300 X vs. uninfected persons….which silences tumor suppressor genes

  Cancer Res 2010;70:1430

EPIGENETICS: ONCOGENIC VIRUSES

- Most frequently studied: HBV, HPV, EBV

- HBV:
  - >2B infections; 350M are chronic
  - Hepatocellular Carcinoma is 100 X higher in HBV infected person

Genome Res 2009;19:438

EPIGENETICS: ONCOGENIC VIRUSES

- **HPV:**
  - **Most frequent infectious agent causing cancer**
  - **Linked to >70% cervical cancers, most anal cancers, respiratory papillomatosis, breast cancer, head-neck cancers**

Head Neck Pathol 2009;3:78

EPIGENETICS: ONCOGENIC VIRUSES

- EBV:
  - First carcinogenic virus identified….
  - Infects >90% humans
  - N-P carcinoma, Burkitt's lymphoma, Hodgkin disease, lymphoproliferative tumors in immunocompromised patients

JAMA 2011;305:1484

ONCOGENIC VIRUS: MECHANISM

- HPV, HBV, EBV
  - Progressive methylation as acute infection becomes chronic infection then premalignant lesion and invasive cancer

    Genome Res 2009;19:43

- The same pathogen can induce several epigenetic modifications resulting in complex “crosstalk”

  Oncogene 2010;29:1405

ONCOGENIC VIRUSES: EXAMPLES

- HTLV-1 induces abnormal methylation on short arm of chromosome II that harbors tumor suppression genes . . . tumor genesis and progression

Proc Natl Acad Sci USA 1988;85:5693

EPIGENETICS: MECHANISM

- Reversible, heritable changes that alter chromatin compaction and gene expression
- Most studied:
  - DNA methylation by covalent linkage to position 5' cytosines within CpG dinucleotides
  - Post-translational histone modifications covalently change N-terminal tail and modify chromatin condensation
  - RNA-mediated silencing (non-coding 19-25 nucleotide RNA molecules) …micro-RNAs targeting up to 1/3 mRNAs in humans → negatively controls gene expression

JAMA 2011;305:1484

EPIGENETICS: "FIELD FOR CANCERIZATION"

- Infection → ↑ DNA methylation → marks region for ↑ RF malignant transformation by silencing tumor suppression genes

- DNA methylation: useful marker for malignant transformation (including breast, liver, colon)

Cancer Res 2010;70:1430

EPIGENETICS: IS THERE A SWITCH?

- Micro-RNAs (mir -21, mir-181b-1): key regulators of tumor-suppressor genes
- Results in positive feedback loop with epithelial cell transformation/propagation

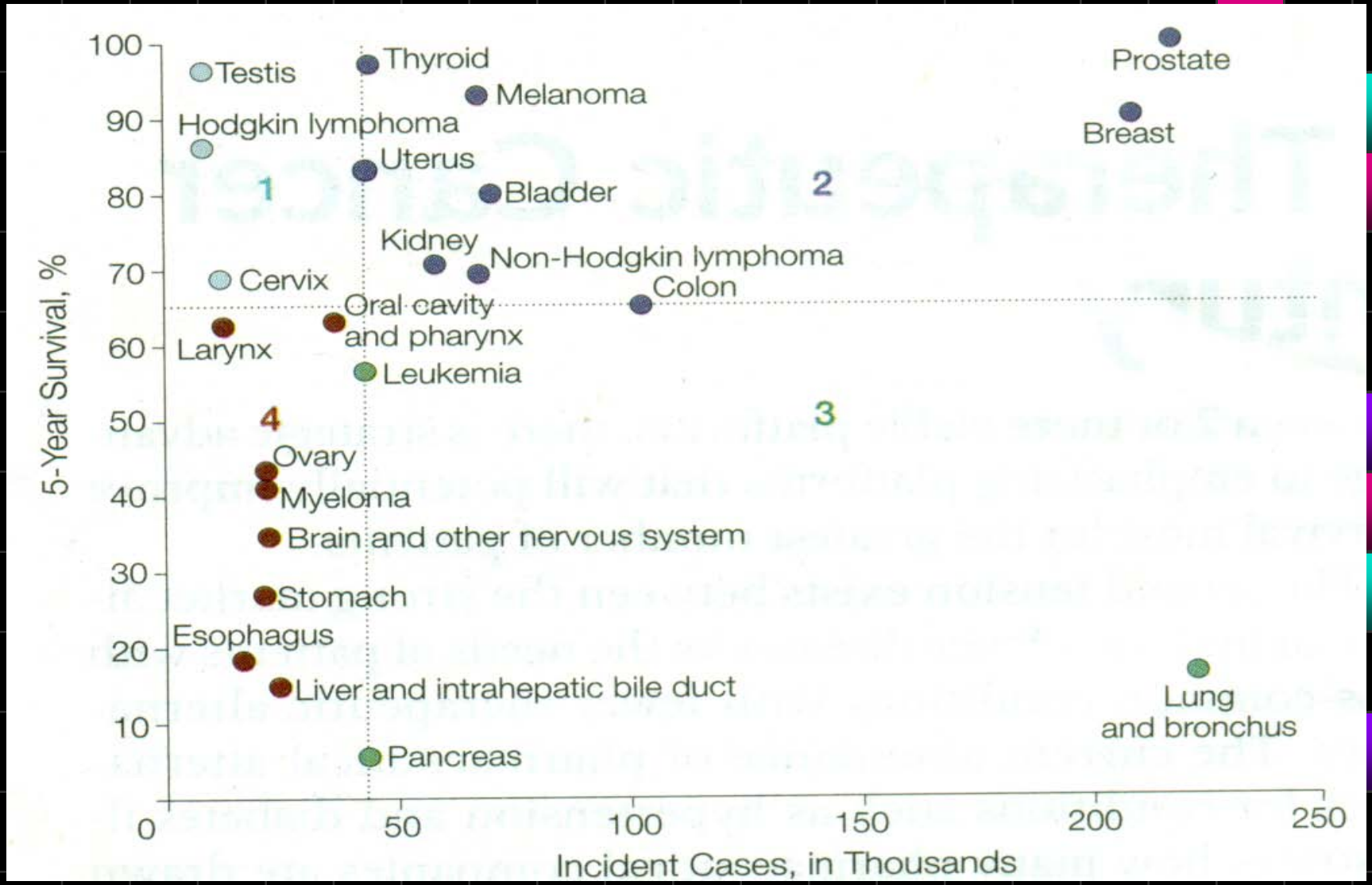
Mol Cell 2010;38:493

JAMA 2011;305:1484

EPIGENETIC SWITCH?

- Can vaccines (immunomodulators) be developed targeting epitopes that act as gateways for these epigenetic modifications?

ANNUAL INCIDENCE AND 5-YEAR SURVIVAL RATES FOR DIFFERENT TYPES OF CANCERS



Therapeutic Cancer Vaccines

- 2010: Sipuleucel-T…First licensed vaccine in the US…refractory Prostate Cancer

- Intrinsic Tensions in Development

  *What is most Scientifically Accessible

  * What is most Needed

JAMA 2011;305:2343

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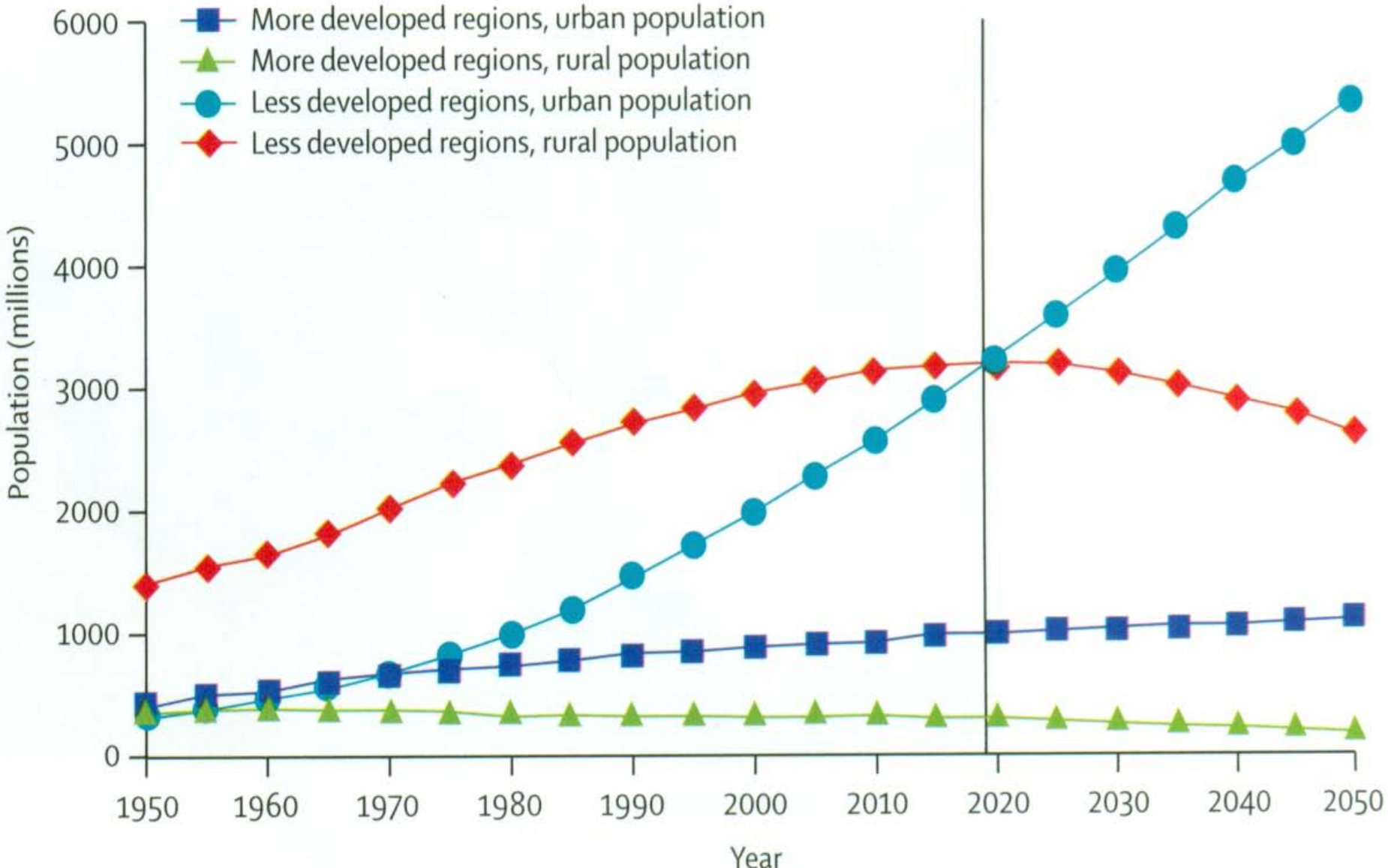
GLOBAL POPULATION SHIFTS

- Urbanization in industrialized nations contributed to overall improvement in population health
- Urbanization
  - 2009: >50% world's population lives in cities
  - Population in urban areas will double (3.3B in 2007 → 6.3B in 2050)
  - Majority will occur in developing countries
- Cities are becoming hubs for transmission of infectious diseases
- Sub-Saharan Africa remains mainly rural with "tipping" (>50%) not expected before 2030

Lancet Inf Dis 2010;10:131

EVOLUTION OF URBAN AND RURAL POPULATIONS BETWEEN 1950 AND 2050



SOCIOECONOMIC DISPARITIES IN PARAISOPOLIS, SAU PAULO

US HOSPITALIZATIONS DUE TO INFECTIOUS DISEASES (ID)

- 1998-2006
  - >40M hospitalizations due to ID
  - 4.5M hospital days
  - $865B hospital charges
  - ↑ From 1998 → 2005 ($p < .001$)
- . . . Implications with an aging population:

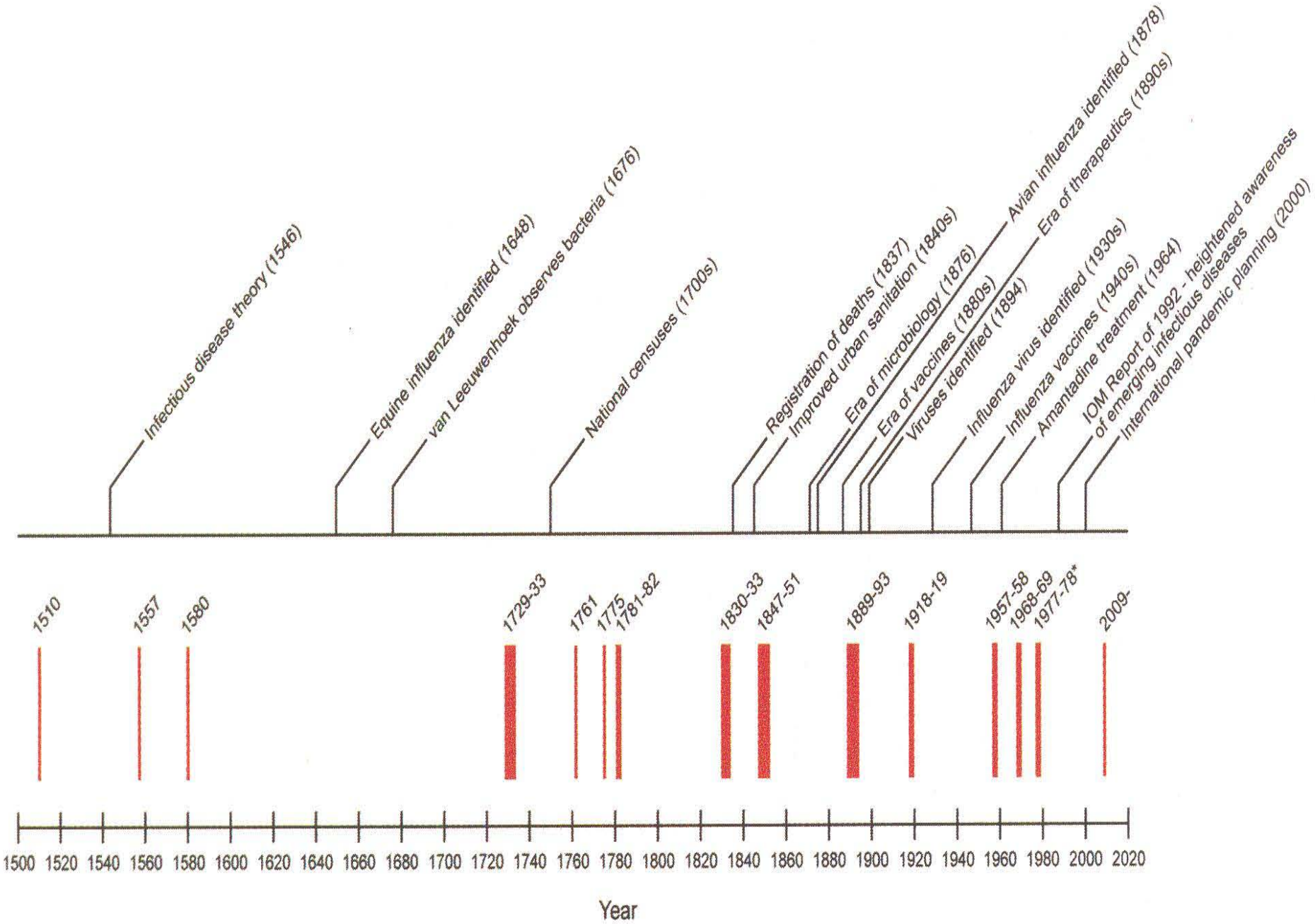
Prevention strategies vital

CID 2009;49:1025

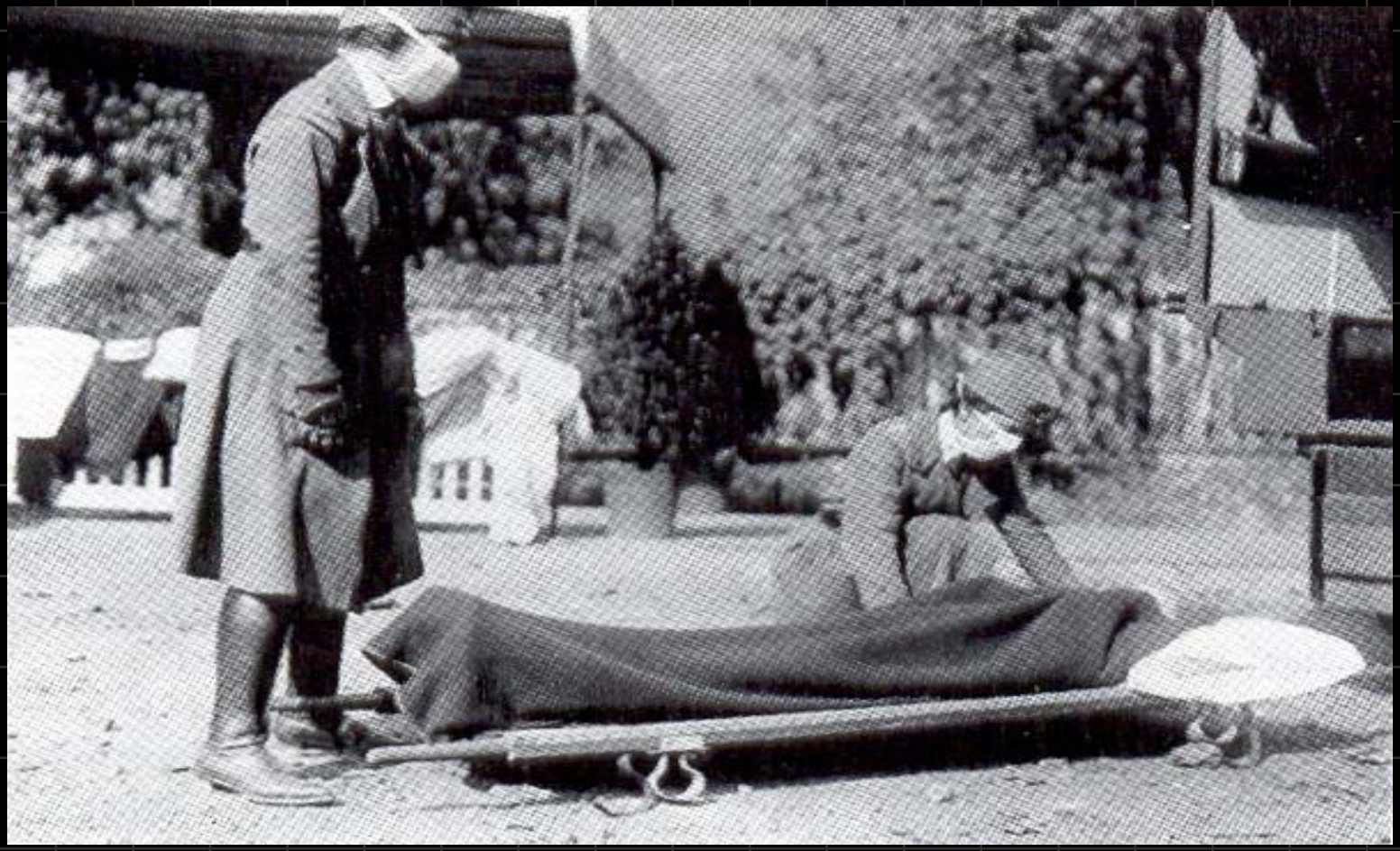
“The only thing new in the world is the history you don’t know.”

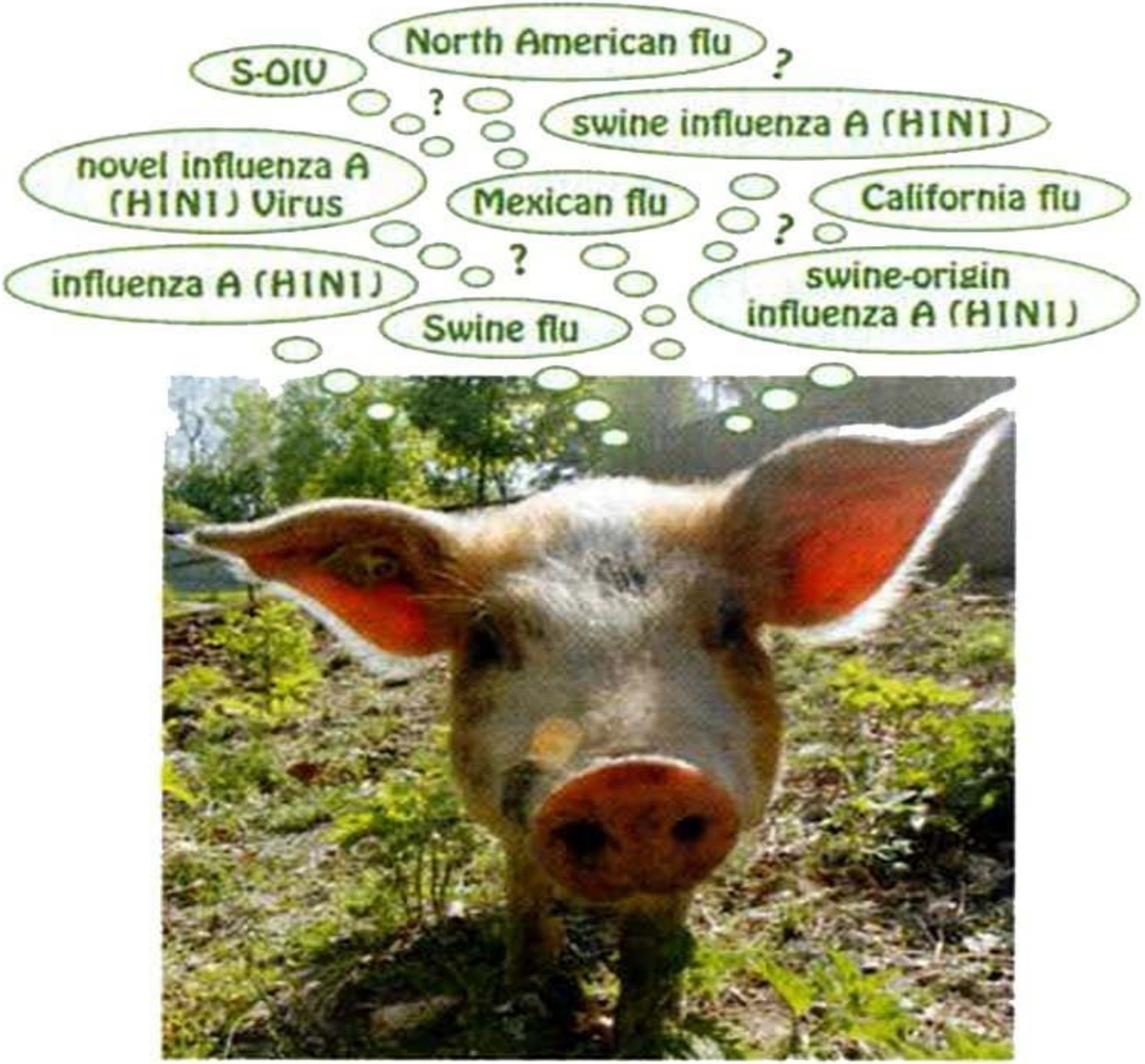
Harry S. Truman

PANDEMIC INFLUENZA'S 500TH ANNIVERSARY



THE INFLUENZA PANDEMIC OF 1918 KILLED 20 MILLION TO 40 MILLION PEOPLE WORLDWIDE




North American flu
S-OIV
swine influenza A (H1N1)
novel influenza A (H1N1) Virus
Mexican flu
California flu
influenza A (H1N1)
swine-origin influenza A (H1N1)
Swine flu

“FACTS DO NOT CEASE TO EXIST BECAUSE THEY ARE IGNORED”

Aldous Huxley

INFLUENZA: EPIDEMIOLOGY

INFLUENZA VACCINE (I-V): HIDDEN EFFICACY

- Influenza responsible for 200K hospitalizations and 36K deaths annually
  - ↑ risk for infants, pregnant women, elderly, chronic illness
  - Among children, ↑ infants < 6 months
  - I-V recommended for infants > 6 months

CID 2010:51:1355

INFLUENZA VACCINE (I-V): HIDDEN EFFICACY (cont.d)

- **Results:**

Infant Age	⊕ I-V	⊖ I-V	P-value
< 6 months	2.2%	19.9%	.001
> 6 months	4.6%	5.6%	NS

CID 2010:51:1355

INFLUENZA VACCINE (I-V): HIDDEN EFFICACY (cont.d)

- Thus: Inactivated influenza vaccine given to pregnant women is highly effective (91.5%) in preventing hospitalizations for laboratory-confirmed influenza among their infants aged <6 months

CID 2010:51:1355

INFLUENZA VACCINE: CHILDREN

- P/Cohort trial (N = 631; range 9-40 months)

	+V	-V	P-value
Influenza (all ages)	5%	13%	.003
Influenza (<2 yrs)	4%	12%	.03

- Thus: Trivalent inactivated vaccine is efficacious

Lancet Inf Dis 2011;11:23

INFLUENZA VACCINE
[2010 : 2011]

- Influenza vaccine: 2010-2011:
- Influenza vaccine: 2011-2012:

  A/California/7/2009 (h1N1)-like virus

  A/Perth/16/2009 (H3N2)-like virus

  B/Brisbane/60/2008-like virus

……Implications and Challenges for 2011

CDC Webpage

VACCINE DEVELOPMENT: INTRADERMAL

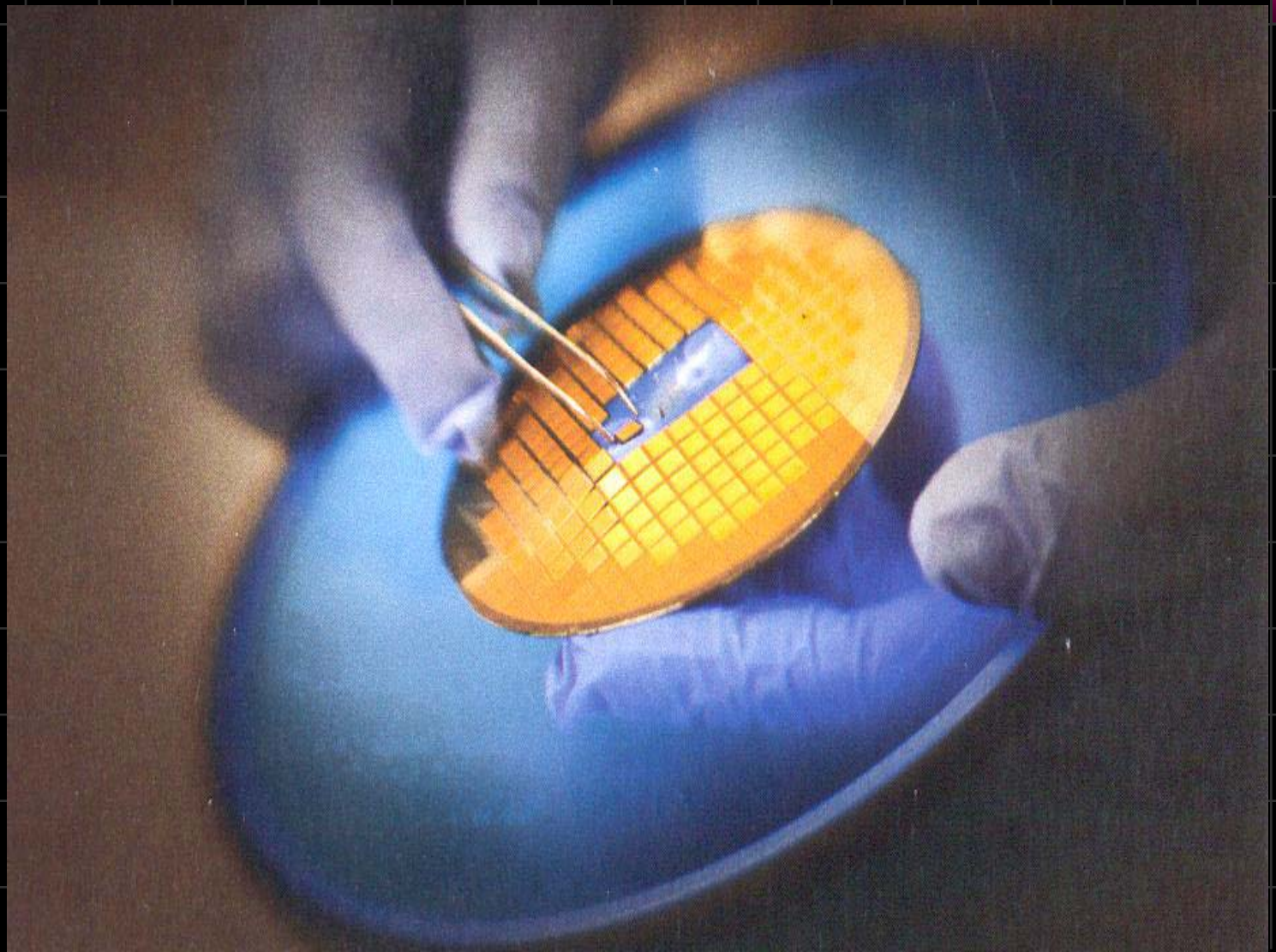
- Although intramuscular injections are traditional vehicles for vaccine delivery, intradermal delivery has been shown to enhance immunogenicity for Hepatitis B, Rabies

Lancet 1983;2:301

Vaccine 1992;10:33

VACCINATION WITH NEEDLES CARRIES RISKS FOR MEDICAL PERSONNEL

NANOPATCH SYSTEMS USE FINE NEEDLES TO DELIVER VACCINES BENEATH THE OUTER LAYER OF SKIN



VACCINE DEVELOPMENT: INTRANASAL

- Intranasal (IN) delivery can induce mucosal immunity
- Advantages:
  - Ease of administration
  - Noninvasive delivery
  - Minimal skill requirements (e.g., mass vaccination scenario)
  - Powder formulations (newer) no longer require refrigeration/frozen storage

JID 2005;191:278

VACCINE DEVELOPMENT: DEPLOYMENT

- If minimally invasive vaccine delivery (skin or nasal) available as unit dose . . .
  - Allows for rapid vaccination by clinic staff
  - ? Self-administration during time of emergency….Disaster Planning

. . . Overcoming vaccine distribution logistics

JID 2005;191:278

VACCINE DEVELOPMENT: INTRANASAL

- Anthrax rPA has been given intranasally to mice in liquid form with complete protection to aerosol challenge

  Inf Imm 2002;70:2022

  J Immun 2003;170:5636
- Anthrax rPA has been given intranasally to rabbits in powder form with complete protection to aerosol challenge

  JID 2005;191:278
- Commonly, new vaccines/new delivery modalities must demonstrate efficacy in two animal models prior to human clinical trials

NANOBODY AS NOVEL THERAPY

- Nanobody (NB)
  - Fragments of single chain variable domain immunoglobulin
  - Easily produced, purified
  - Can be engineered into multiple configurations (bivalent, pentavalent constructs)
  - Intranasal introduction can introduce NB deeply into respiratory tract
- Mice model protected by llama-derived NB when challenged with H5N1 influenza virus

JID 2011:203:1063

VACCINE DEVELOPMENT: ANTHRAX

- Intramuscular injection
- Microneedle intradermal
- Inhalation liquid
- Inhalation powder
- Epidermal microabrasion

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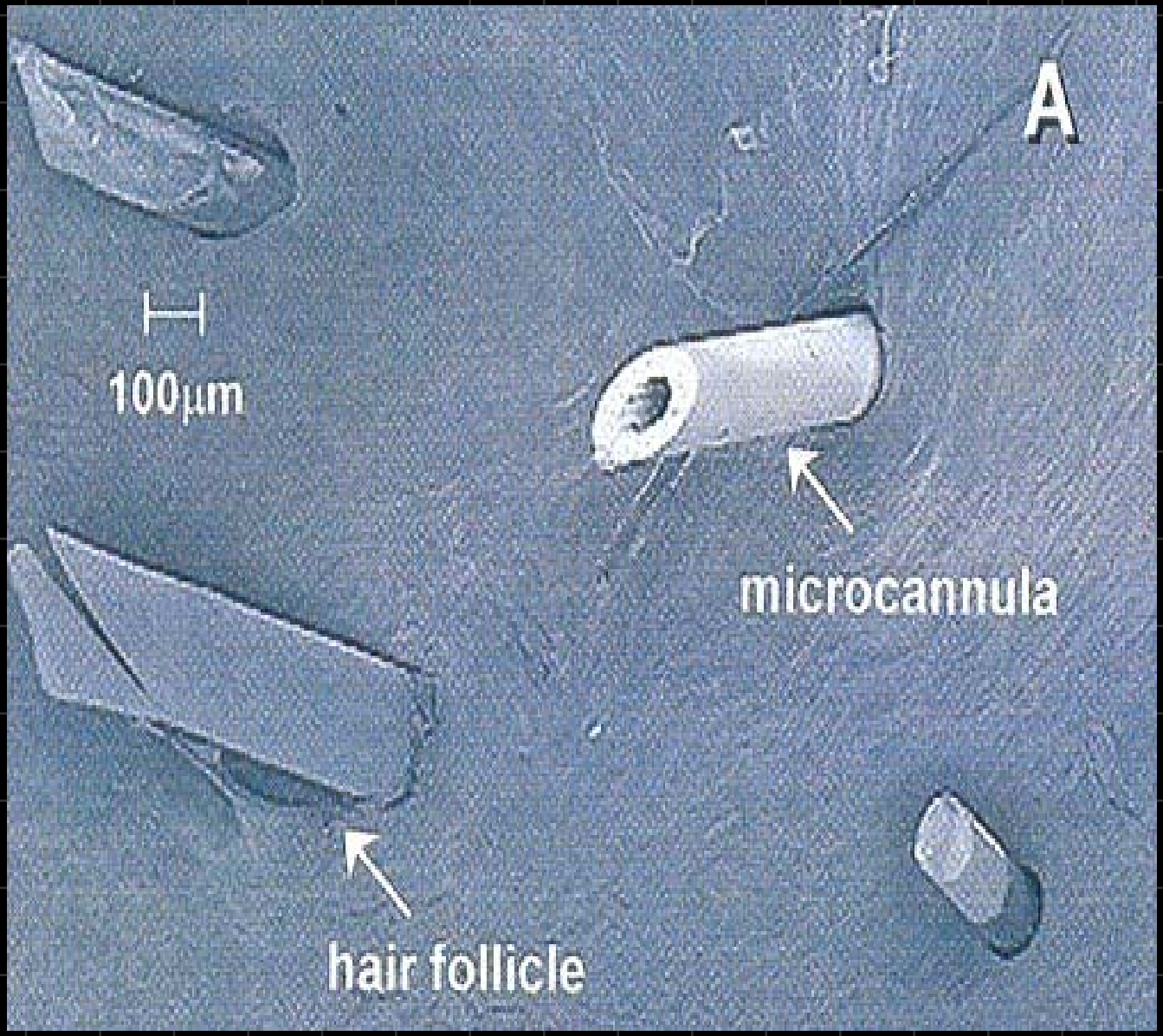
A
100μm
microcannula
hair follicle

VACCINE DEVELOPMENT: ANTHRAX

- Intramuscular injection
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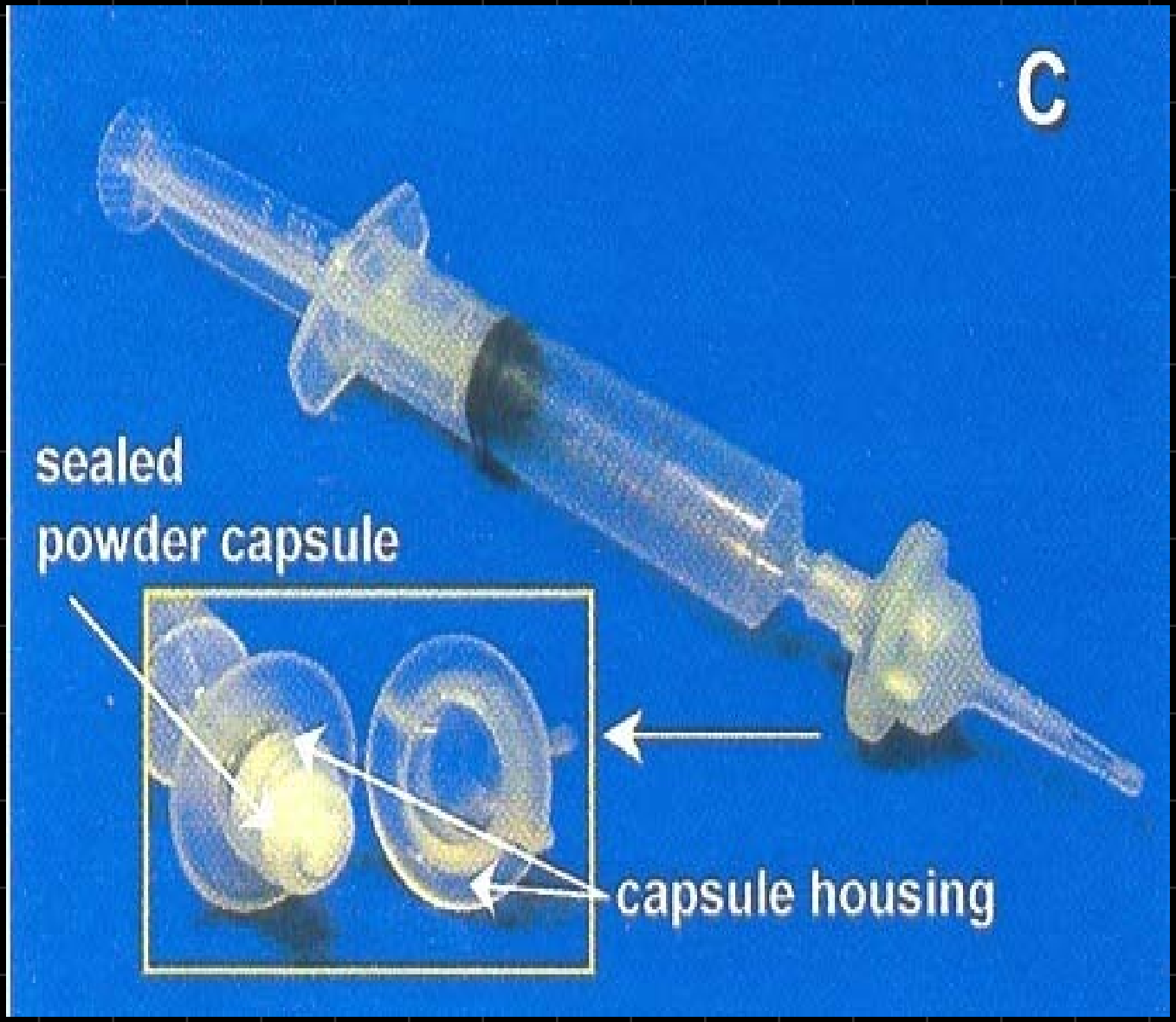
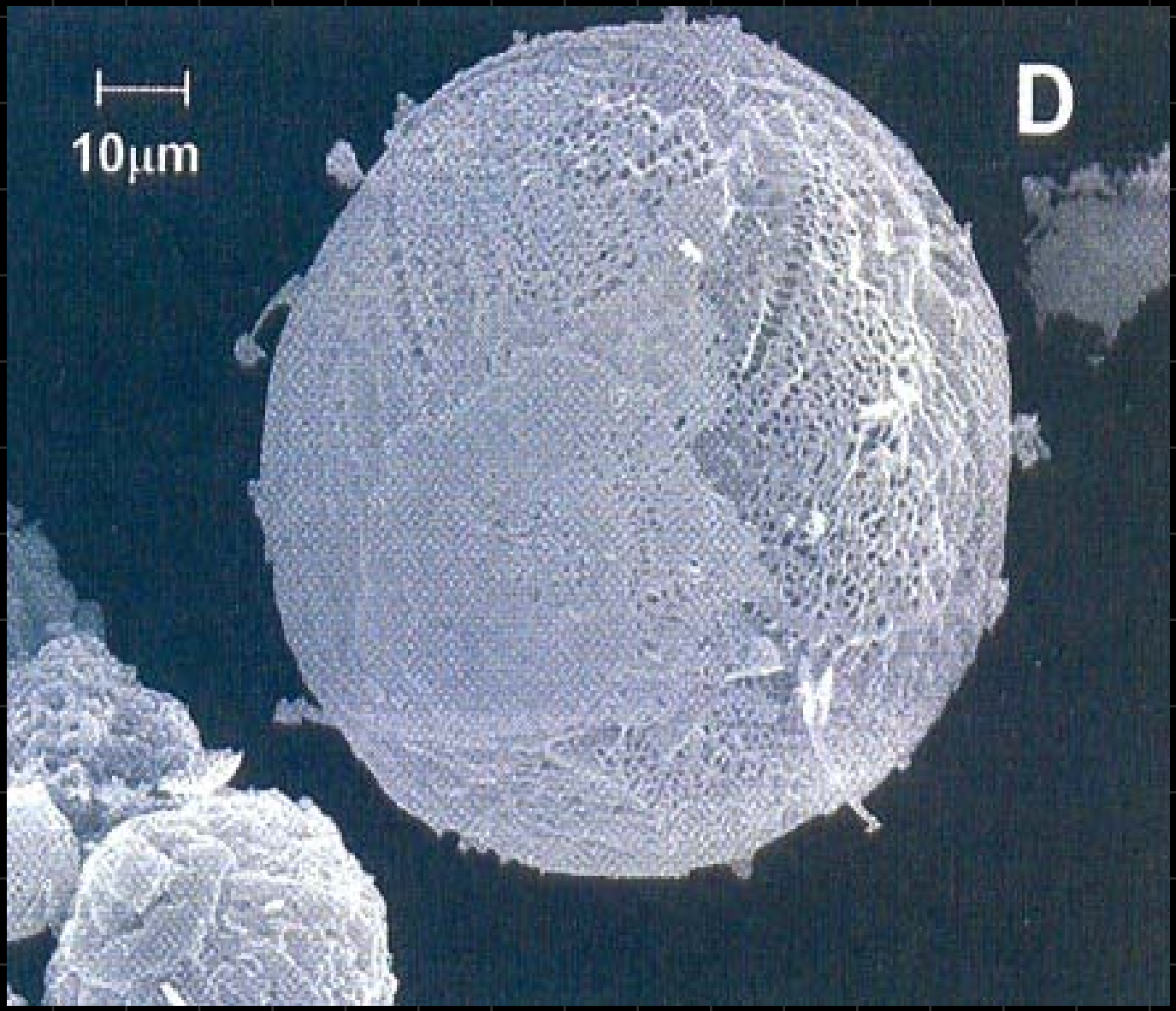
- <u>Inhalation liquid</u>
- Inhalation powder
- Epidermal microabrasion


C
sealed
powder capsule
capsule housing

VACCINE DEVELOPMENT: ANTHRAX

- Intramuscular injection
- Microneedle intradermal
- Inhalation liquid
- <u>Inhalation powder</u>
- Epidermal microabrasion


10μm
D

VACCINE DEVELOPMENT: ANTHRAX

- Intramuscular injection
- Microneedle intradermal
- Inhalation liquid
- Inhalation powder
- <u>Epidermal microabrasion</u>

B
200µm

VACCINE DEVELOPMENT: INTRADERMAL

- Skin possesses strong immunostimulatory properties
  - Contains abundance of antigen-presenting cells (APC)
  - Conventional skin needle delivery systems are difficult to master (size, depth, bevel angle)
  - Subject to clinical variability

J Control Release 2000;66:199

VACCINE DEVELOPMENT: INTRADERMAL

- Alternative delivery systems
  - Jet injection
  - Iontophoresis
  - Laser induction
  - Sonophoresis
  - Electroporation
  - Microporation

JID 2005;191:278

VACCINE DEVELOPMENT: INTRADERMAL

- Specialized formulations designed to breach skin barrier
  - Bacterial enterotoxins
  - Viral vectors
  - Liposomes
  - Nanoparticles coupled with APC-targeting molecules
- Example: E. coli enterotoxin impregnated skin patch attached to recombinant protective antigen of anthrax (rPA)
  - Formulation conferred complete protection against subcutaneous anthrax challenge in rabbit

Infect Immun 2004;72:1181

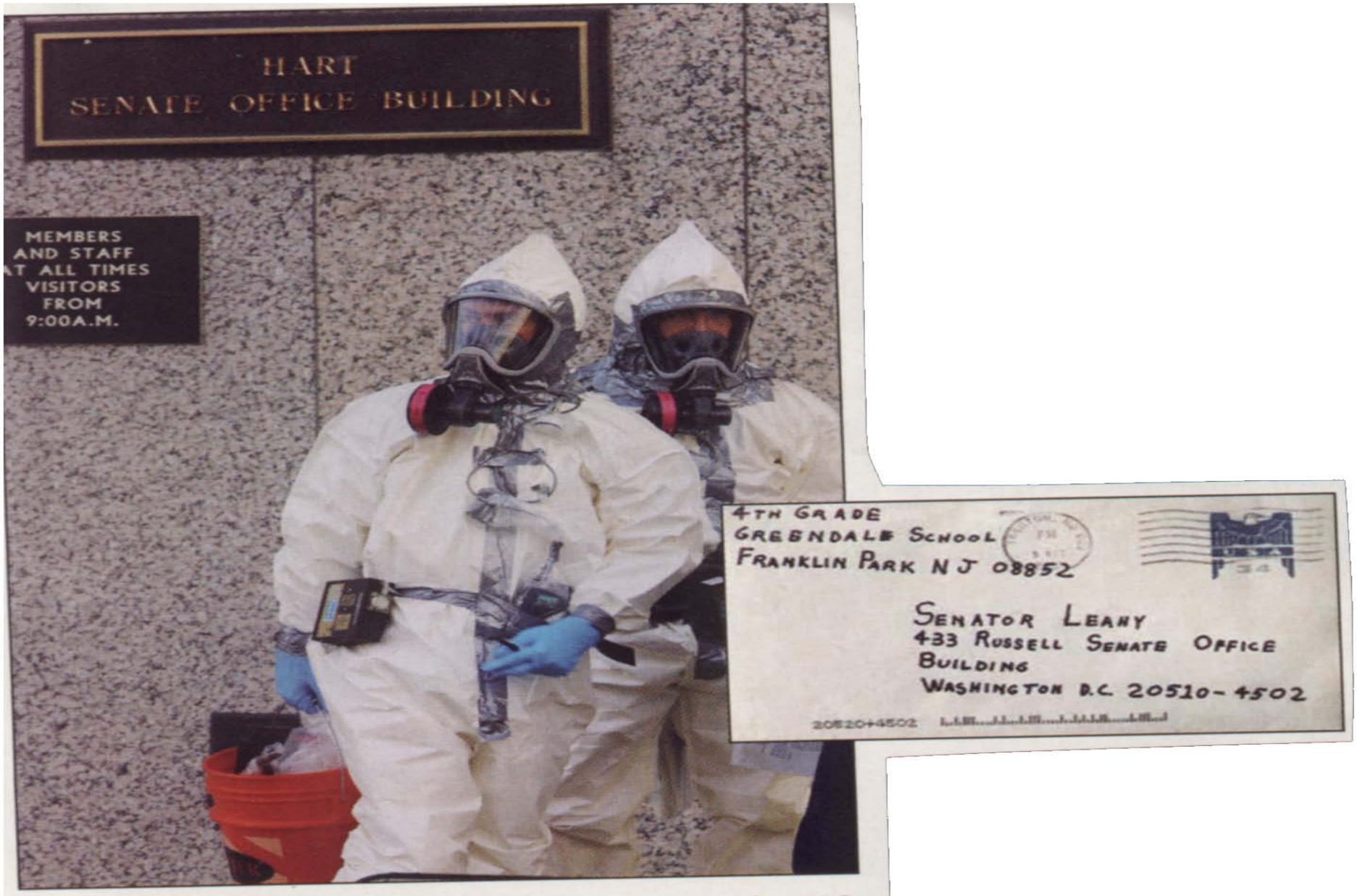
DISASTER PLANNING

- Disaster:
  - Latin derivative of astrum or star
  - A calamity visited upon mankind must be due to a misalignment of the stars

“…ring around the roses,
a pocket full of posies,
ashes, ashes, we all fall down…”

The Decameron of Boccaccio circa 1566

* > 100,000 deaths from plague in Florence, Italy



Rapid release. The powder in letters sent to the U.S. Senate was treated in a sophisticated way to create an aerosol, some researchers say.

ANTHRAX 2001: CLINICAL SCENARIOS

- 11 patients documented during October-November, 2001
- Work characteristics
  - 9/11 processed/screened letters containing B. anthracis spores (6 postal workers, 2 mail sorters, one journalist)
  - 2/11 thought to have been exposed to spores from cross-contaminated mail
  - 1/11 known occupational risk

CID 2003; 36 (10):1275-1283

BIOTERRORISM: ANTHRAX

- **Causative agent:**
  - **Spore-forming, gram-positive *Bacillus anthracis***

- **Incubation**
  - **Inhalation = 2 - 60 days**
  - **Ingestion/cutaneous ≅ 1 - 7 days**

ANTHRAX: INTERVENTION

- Vaccine
  - Inactivated, cell-free product (1970)
  - Given as a 6-dose series
  - Mandated for active/reserve US military
  - Approx. 600K doses given by 1999
  - No causally-related adverse events documented
  - Efficacy (monkeys) after aerosol challenge 100% at 8/38 weeks, 88% at 100 weeks

Salisbury Med Bull 1996;87:125

ANTHRAX VACCINE: PREGNANCY

- Study to assess safety of product

	Vaccinated	Unvaccinated
# Vaccines given	7,464	---
# Pregnancies	385	130
# Person-months follow-up	28,815	9,734
Pregnancy rate (per 1000 person-yrs)	159.5	160

- No increased risk for low birth weight, structural/cosmetic abnormalities

JAMA 2002;287:1556

ANTHRAX VACCINE

- Unclear duration of spore survival in lungs even after antibiotic prophylaxis
- Focus of concern: Re-aerosolization
- Post-exposure prophylaxis (PEP) options:
  - Standard PEP = 60 days
  - Extended PEP = 100 days
  - Vaccine (3 doses over 4 weeks) + ABX
- Pre-exposure vaccination reserved for:
  - Military personnel
  - Certain laboratory workers

Inf Dis News 2002;15:6

Flagellants at the Time of the Black Death

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STAPHYLOCOCCUS AUREUS: HISTORY

- 1941: Albert Alexander (Constable, Oxfordshire, England) first recorded recipient of penicillin
- 1942: Remmelkamp reported first clinical isolates of penicillin Ⓡ Staphylococcus aureus
- 1960: Methicillin introduced
- 1961: MRSA first reported

Proc Royal Soc Exper Biol Med 1942;51:386

MRSA: COMMUNITY-ACQUIRED CASES

- CA-MRSA first reported (1999) in four fatal pediatric cases
- Pediatric cases carry Panton-Valentine Leukocidin (PVL) toxin
- Majority of CA-MRSA are skin/skin structure but 5% bacteremia
- Adults may possess antibody to PVL toxin (Thus, ↓ affected)

ICAAC 2003; Session 59

S. AUREUS (SA) INFECTION (1998-2003)

- Rate of SA infection:
  - ↑ from 0.7% → 1.0% for all hospitalizations (p=.004)
  - ↑ from 0.9% → 1.3% for all surgical hospitalizations (p=.001)
  - ↑ from 1.2% → 1.8% for all orthopedic hospitalizations (p=.001)
- Economic burden increased from 9.2% → 17.9% (p<.05)

CID 2007;45:1132

MRSA “SPIDER BITE”

S. AUREUS (SA): COLONIZATION

- National estimates
  - 32% of people colonized with SA
  - 0.8% of people colonized with MRSA

Ann Int Med 2006;144:318

S. AUREUS (SA) SCREENING: WHERE ?

- Anterior nares is the most important screening site for SA

  . . . but . . .

- Results: (N=2966)
  - Both nares = 37% Cx ⊕
  - Throat Cx only = 12.8% Cx ⊕
  - Either = ~ 50%

Thus, throat swab screening ↑ sensitivity of SA detection by 25.7%

CID 2007;45:475

MRSA Ward →
← MRSA Wing
MRSA Waiting Room →
EXIT

MRSA NASAL CARRIAGE: INFECTION RISK

- Meta-analysis; 10 studies (n = 1170)

	After MRSA	After MSSA
Invasive infection after colonization	4 x	x

- Implications . . .
  - Case finding
  - Decolonization
  - ? Strain virulence

Am J Med 2008;121:310

S. AUREUS SKIN INFECTIONS: OUTCOMES

- Prospective outcomes study @ 30D, 120D, after diagnosis
-

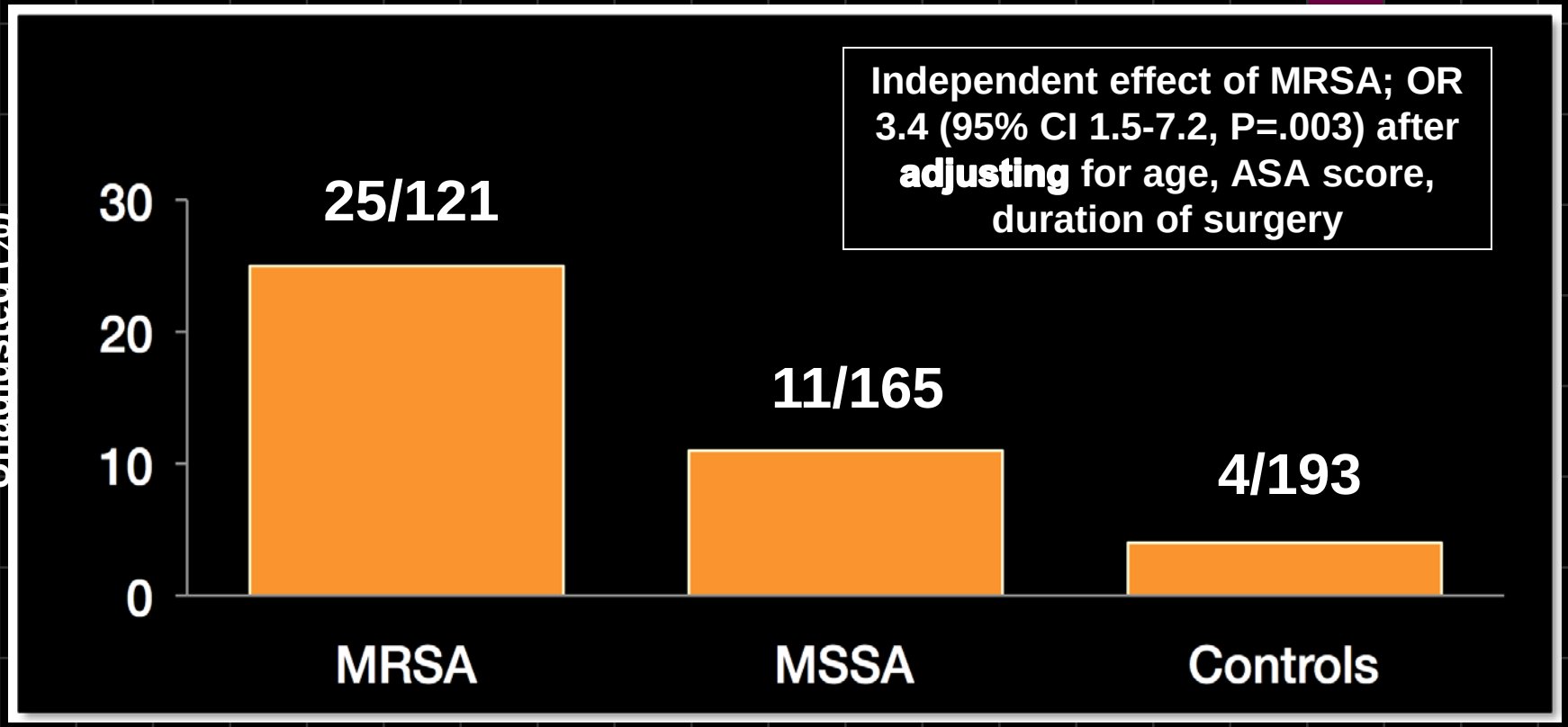
	Co-MSSA	Co-MRSA	p-value
Skin infection (N)	47	70	--
Nonresponse			
@ 30D	28%	33%	NS
% Re-hospitalization	23%	4%	0.003

- Those patients who did not receive I&D procedure were ↑ nonresponders at day 30 for (both) MSSA/MRSA; p = .005

CID 2007:44:483

Staphylococcus aureus Surgical Site Infections (SSI)

Impact of Methicillin Resistance on Outcomes



MRSA=methicillin-resistant *Staphylococcus aureus*; MSSA=methicillin-susceptible *Staphylococcus aureus*; OR=odds ratio; ASA Score=American Society of Anesthesiologists Score of pre-operative physical fitness.

Engemann JJ, Carmeli Y, Cosgrove SE, et al. Adverse clinical and economic outcomes attributable to methicillin resistance among patients with *Staphylococcus aureus* surgical site infection. *Clin Infect Dis.* 2003;36;592-598.