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MEDICAL NOTES POWERHOUSE

Medical Notes Powerhouse

The Complete High-Yield Notes for NEET SS & INI SS-ET

Infectious Disease I
Bacteria

03 VOLUME
of 10 · Volume III · CH 124–186

56 CHAPTERS

5 SECTIONS

10 VOLUME SET

SECTIONS IN THIS VOLUME

- Principles of ID
- Bacterial Syndromes
- Gram-Positive Bacteria
- Gram-Negative Bacteria
- Mycobacteria & Atypicals

Sources Harrison's Principles of Internal Medicine, 22nd Edition

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Table of Contents

Medical Notes Powerhouse · Volume III · 56 chapters · 5 sections

SECTION I
Principles of Infectious Disease
7 chapters

124 **Approach to Infectious Disease** 1
History, Exam, Diagnostics

125 **Molecular Microbial Pathogenesis** 5
Entry, Attachment, Virulence

126 **Microbial Genomics & ID** 10
NAATs, Resistance, Genomics

127 **Acutely Ill Febrile Patient** 13
ID Emergencies: Recognition & Rx

128 **Principles of Immunization** 17
Vaccine Types, Immunology

129 **Vaccine Use in Adults** 21
Schedule, Contraindications

130 **International Travel Health** 25
Vaccines, Malaria Prophylaxis

SECTION II
Bacterial Infections by Syndrome
18 chapters

131 **Pneumonia** 30
CAP · HAP · VAP · Aspiration

132 **Lung Abscess** 35
Necrosis + Cavitation of Lung

133 **Infective Endocarditis** 38
Duke-ISCVID · Empirical & Targeted Rx

134 **Skin & Soft Tissue Infections** 44
Anatomic Approach

135 **Infectious Arthritis** 49
Bacterial · Gonococcal · TB · Fungal

136 **Osteomyelitis** 54
Vertebral · Long Bone · PJI · Sternal

137 **Intraabdominal Infections** 59
Peritonitis · Abscesses

138 **Acute Infectious Diarrhea** 63
Pathogens · Mechanisms · Rx

139 **C. difficile Infection** 68
Pseudomembranous Colitis · FMT

140 **Urinary Tract Infections** 72
Cystitis · Prostatitis · Pyelonephritis

141 **Sexually Transmitted Infections** 77
Overview & Clinical Syndromes

142 **Encephalitis** 83
Acute & Chronic Viral Encephalitides

143 **Acute Meningitis** 88
Bacterial · Viral · Subacute

144 **Chronic & Recurrent Meningitis** 94
Approach · Etiology · Workup

145 **Brain Abscess & Empyema** 99
Focal Suppurative CNS Infections

146 **Infectious Complications of Bites** 104
Dog · Cat · Human · Snake

147 **Healthcare-Acquired Infections** 108
Surveillance · Device-Related

148 **Infections in Transplant Recipients** 112
HCT · SOT · Vaccination

SECTION III
Gram-Positive Bacteria 4 chapters

156 **Listeria monocytogenes Infections** 118
Listeriosis — Foodborne

157 **Tetanus** 122
C. tetani — Spastic Paralysis Toxin Disease

158 **Botulism** 126
BoNT — Descending Flaccid Paralysis

159 **Gas Gangrene & Clostridial Infections** 130
Histotoxic Clostridia

SECTION IV
Gram-Negative Bacteria 19 chapters

160 **Meningococcal Infections** 135
N. meningitidis — Septicaemia & Meningitis

161 **Gonococcal Infections** 140
N. gonorrhoeae — STI, DGI & Ophthalmia

162 **Haemophilus & Moraxella Infections** 144
H. influenzae, M. catarrhalis

163 **HACEK Group & Misc Gram-Negatives** 149
Fastidious GNRs

164 **Legionella Infections** 153
Legionnaires' Disease & Pontiac Fever

165 **Pertussis & Bordetella** 157
Whooping Cough — 100-Day Cough

166 **Gram-Negative Enteric Bacilli** 161
Enterobacterales — ESKAPE

167 **Acinetobacter Infections** 167
A. baumannii — ICU & War Wounds

168 **Helicobacter pylori Infections** 171
PUD, Gastric Cancer Risk

169 **Campylobacter & Related Organisms** 175
C. jejuni Enteritis

170 **Pseudomonas, Burkholderia & Stenotrophomonas** 179
Non-Fermenters

171 **Salmonellosis** 183
Enteric Fever & Nontyphoidal Salmonella

172 **Shigellosis** 188
Bacillary Dysentery — Shigella spp.

173 **Cholera & Other Vibrioses** 191
V. cholerae · V. parahaemolyticus

174 **Brucellosis** 195
Undulant Fever — Zoonotic Brucella spp.

175 **Tularemia** 199
Francisella tularensis — Rabbit/Deer-Fly Fever

176 **Plague & Yersinia Infections** 203
Y. pestis · Y. enterocolitica

177 **Bartonella Infections** 207
CSD · Trench Fever · Bacillary Angiomatosis

178 **Donovanosis** 211
Granuloma Inguinale — Klebsiella granulomatis

SECTION V
Mycobacteria & Atypicals 8 chapters

179 **Nocardiosis** 214
Nocardia spp. — Pulmonary · CNS · Cutaneous

180 **Actinomycosis** 218
Actinomyces spp. — Most Misdiagnosed Infection

181 **Whipple Disease** 222
Tropheryma whipplei — Chronic Multisystem

182 **Mixed Anaerobic Infections** 226
Bacteroides · Prevotella · Fusobacterium

183 **Tuberculosis** 230
M. tuberculosis — Pulmonary · Extrapulmonary

184 **Leprosy** 236
Hansen Disease — M. leprae · Ridley-Jopling

185 **NTM Infections** 241
MAC · M. kansasii · M. abscessus

186 **Antimycobacterial Agents** 245
First-line · Second-line · Novel

End of Table of Contents

SECTION I

Principles of Infectious Disease

Pathogenesis, microbial genomics, immunisation and travel medicine

IN THIS SECTION — 7 CHAPTERS

124 **Approach to Infectious Disease**
History, Exam, Diagnostics

125 **Molecular Microbial Pathogenesis**
Entry, Attachment, Virulence

126 **Microbial Genomics & ID**
NAATs, Resistance, Genomics

127 **Acutely Ill Febrile Patient**
ID Emergencies: Recognition & Rx

128 **Principles of Immunization**
Vaccine Types, Immunology

129 **Vaccine Use in Adults**
Schedule, Contraindications

130 **International Travel Health**
Vaccines, Malaria Prophylaxis

CH 124 · Approach to Infectious Disease — History, Exam, Dia...

Framework for the febrile/infected patient: focused history (exposure + host), exam pearls (Faget sign, lymphadenopathy, skin), key diagnostics (CSF, WBC, ESR/CRP), empirical antibiotic Rx.

Scope & Global Burden

- Infectious diseases (ID) = **2nd leading cause of death worldwide**.
- 2019 → **9.6 million deaths** globally from ID (~17% of all deaths; 69% in sub-Saharan Africa).
- Disproportionately affects: **infants <1 y, adults >70 y**, low/middle-income countries.
- **~16% of all malignancies** linked to infection — H. pylori (gastric Ca), HPV (cervical Ca), HBV/HCV (liver Ca).
- Global travel + migration → rapid spread (SARS-CoV-2 = ~7 million deaths).
- Escalating **AMR**: CRE, *Acinetobacter*, *Candida auris*, MDR-TB, VRE.

Microbiota & Pathogens

- **~40 trillion** microbes colonize healthy host — outnumber human cells ~10×.
- Major reservoir = **GI tract**; also genital, oral, nasopharynx, skin, lungs.
- Most infections = from **normal microbiota** (*S. aureus*, *S. pneumoniae*, *P. aeruginosa*).
- Few = strict pathogens (*N. gonorrhoeae*, rabies).
- Microbiota shapes immunity + vaccine response.

History — Exposure

Prior infections / drug resistance

- Prior MRSA, VRE, ESBL, carbapenemase → guides empirical Rx.
- Recent hospital / nursing home / LTAC stay → risk for MDR organisms.

Social & dietary

- High-risk behaviors: unsafe sex, IV drug use.
- Hobby: gardening → *Sporothrix schenckii*.
- Occupation: funeral worker → TB; lab worker → *Y. pestis* (2009 case).
- **Diet cues:**
- **Raw/undercooked meat:**
  - Shiga-toxin *E. coli*, *Toxoplasma*.
- **Unpasteurized milk:**
  - *Salmonella*, *Listeria*, *M. bovis*.
- **Unpurified water:**
  - *Leptospira*, parasites, enterics.
- **Raw seafood:**
  - *Vibrio*, norovirus, helminths, protozoa.

Animal exposures

Table 124-A — Animal → Pathogen

Animal	Pathogen / Disease
Dogs (ticks)	Lyme, RMSF, ehrlichiosis
Cats	*Bartonella henselae*
Reptiles	*Salmonella*
Rodents	Leptospirosis
Rabbits	Tularemia

Travel

- Recent foreign travel → broadens differential (Chap 130).
- Remote travel still relevant — latent TB, *Strongyloides*.
- Domestic geographic exposure: California → *Coccidioides*; Martha's Vineyard → *F. tularensis*.
- Ask about activities (food/water, swim, animals) + immunizations + prophylaxis.

Host-Specific Factors

- Determine immune status — opportunists (*Pneumocystis*, *Aspergillus*, JC virus) target immunocompromised.
- **Causes of immune defect:**
- **Disease:**
  - Malignancy, HIV, malnutrition.
- **Drugs:**
  - Chemo, steroids, biologics (mAbs).
- **Treatment:**
  - TBI, splenectomy.
- **Primary immunodeficiency.**
  - Congenital.
- Always review **immunization record**.

Physical Exam

Vital signs — fever

- Old "normal" 37°C (98.6°F) — based on axillary, now outdated.
- **Rectal > oral > axillary:** rectal +0.4°C above oral, +0.8°C above axillary.
- Temp ↑ later in day, ↑ in women + young.
- Average body temp ↓ **~0.03°C/decade** since 1800s → new normal ~36.7°C.
- **Fever (FUO criterion) = core ≥38.3°C (≥101°F).**
- Every **1°C ↑ → HR ↑ ~10 bpm**.
- **Faget sign** = relative bradycardia (HR lower than expected for temp).

♥ NEET SS HIGHLIGHTS

♥ **Faget sign** = pulse–temperature dissociation → think **intracellular organisms**.
♥ **Typhoid (S. typhi)**, brucellosis, Q fever, leptospirosis, Legionella, Mycoplasma.
♥ Tick-borne: Rickettsia, scrub typhus (*Orientia*), Babesia.
♥ Viral: yellow fever, dengue, viral hemorrhagic fevers (early Marburg/Ebola), viral myocarditis.
♥ Others: diphtheria, malaria.
♥ Non-infectious: drug fever, β-blocker, CNS lesion, lymphoma, factitious.

Lymphatics

- **~600 lymph nodes** in body.
- Normal node = **<1 cm**.
- Describe: location, size, tender, consistency (soft/firm/rubbery), matted.
- Small + firm = **"shotty"** (buckshot pellets).
- **Palpable epitrochlear = always pathologic.**
- Distribution: 75% localized, 25% generalized.
- Localized — head/neck 55%, inguinal 14%, axillary 5%.

Skin

- Full exam — front + back; pressure ulcer = missed cause of ICU FUO.
- **Endocarditis stigmata**: splinter hemorrhages, **Janeway lesions** (painless palms/soles), **Osler nodes** (painful pulp).

Foreign bodies

- Epithelial barrier breach → microbiota gain access to new site.
- Document all lines, tubes, drains: IV, surgical drain, ETT, Foley.

Diagnostics

WBC count

- **Bacterial → PMN ↑ + bands.**
- **Viral → lymphocytes ↑** (or leukopenia).
- **Parasites → eosinophilia.**

❦ CLINICAL PEARLS

❦ **Eosinophilia + CNS:** *Angiostrongylus*, *Gnathostoma*, *Baylisascaris*.
❦ **Eos + lung:** *Strongyloides*, *Toxocara*, *Paragonimus*, *Coccidioides*, *Pneumocystis*.
❦ **Eos + liver:** *Schistosoma*, *Fasciola*, *Clonorchis*, *Opisthorchis*.
❦ **Eos + gut:** *Ascaris*, *hookworm*, *Trichuris*, *Capillaria*, *Anisakis*.
❦ **Eos + bladder:** *S. haematobium*.
❦ **Eos + muscle:** *Trichinella*.
❦ **Eos + lymphatics:** *Wuchereria bancrofti* (filariasis).
❦ **Categories:** mild 500–1500, moderate 1500–5000, extreme >5000 cells/μL.

Inflammatory markers

- **ESR** = indirect, slow → repeat ≤weekly.
- **CRP** = direct, fast → daily measurements useful.
- **ESR >100 mm/h → 90% PPV for serious disease.**
- Procalcitonin, serum amyloid A — emerging, validation pending.

Table 124-3 — ESR >100 mm/h causes

Category	Examples	% Cases
Infection	SBE, abscess, OM, TB, UTI	35–40
Inflammatory	GCA, RA, SLE	15–20
Malignancy	Myeloma, leukemia, lymphoma, Ca	15–20
Other	Drug fever, ischemia, renal	20–35

CSF analysis

- Always: **opening pressure**, cell count, Gram + culture, glucose, protein.
- Gram stain needs **>10^5 bacteria/mL** for reliable positivity; specificity ~100%.
- Bacterial antigen tests → **not** for screening (no better than Gram).
- **Cryptococcal antigen** + serology (*T. pallidum*, *Coccidioides*) — high sensitivity.
- **PCR** — rapid, sensitive (HSV, enterovirus, meningococcus, pneumococcus, mycobacteria) but no resistance profile.

Table 124-4 — CSF profiles (key cutoffs)

Type	WBC/μL	Cell	Glucose	Protein	Common
Normal	<5	Lymph	40–85	15–45	—
Bacterial	>1000	↑↑ PMN ≥80%	<40	>100	*S. pneumo*, *N. mening*
Viral	25–500	Lymph	Normal	20–80	Enterovirus
Fungal	40–600	Lymph/PMN	↓ to N	150–300	*Cryptococcus*, *Candida*
Parasitic	150–2000	↑↑ **Eos ≥50%**	Normal	50–200	*Angiostrongylus*
TB	25–100	Lymph	<50 (75%)	100–200	*M. tuberculosis*
Encephalitis	50–500	Lymph	Normal	50–100	HSV, enterovirus, rabies

♥ NEET SS HIGHLIGHTS

♥ **Eosinophilic meningitis = parasitic** (Angiostrongylus #1).
♥ **Low CSF glucose** → bacterial, TB, fungal (Listeria, neoplastic).
♥ Bacterial OP >300 mmH_2O; normal 50–180.
♥ TB CSF — lymph + low glucose in 75%; acid-fast stain on pellicle ↑ yield.

Cultures & pathogen tests

- Collect **before antibiotics** when feasible.
- If not — Gram stain / KOH prep critical.
- Culture → ID, susceptibility, isolate typing.

- Contaminant vs pathogen — interpret with host + microbiota context.
- **Universal PCR, shotgun metagenomics** — detect non-cultivable organisms; significance evolving.

Empirical Antibiotic Therapy

Table 124-5 — Empirical Rx by syndrome (high yield)

Syndrome	Pathogens	Regimen
Septic shock	*S. aureus*, *S. pneumo*, GNB	**Vanco 15 mg/kg q12h + antipseudomonal β-lactam** (pip-tazo / imipenem / meropenem / cefepime)
Meningitis	*S. pneumo*, *N. mening*	**Vanco + ceftriaxone 2g q12h**; **dexa 0.15 mg/kg q6h × 2–4d** 10–20 min before 1st abx if pneumococcal
CNS abscess	Strep, Staph, anaerobes, GNB	**Vanco + ceftriaxone + metronidazole 500 mg q8h**
Acute endocarditis (native)	*S. aureus*, strep, CoNS	**Vanco + cefepime 2g q8h**
CAP outpatient, no comorbid	*S. pneumo*, *Mycoplasma*, *H. flu*, *C. pneumo*	**Azithromycin** 500→250 mg PO ×5d
CAP outpatient, comorbid	Same + DRSP	**Levofloxacin 750 mg PO qd**
CAP inpt, non-ICU	+ *Legionella*	Resp FQ **OR** β-lactam + azithro
CAP ICU	+ *S. aureus*	β-lactam + (azithro OR resp FQ)
HAP/VAP	GNB (*Pseudo*, *Kleb*, *Acineto*), *S. aureus*	Antipseudo β-lactam + FQ or aminoglycoside; **add vanco/linezolid if MRSA**
IAI mild-mod	Anaerobes, GNB, strep	Cefoxitin **OR** metronidazole + (cefazolin/cefuroxime/ceftriaxone/cipro/levo)
IAI high risk	Same	**Carbapenem** OR pip-tazo OR metro + antipseudo cephalosporin

CLINICAL PEARLS

- **Daptomycin NOT for pneumonia** — surfactant inactivates it.
- **Dexamethasone** in pneumococcal meningitis — give **10–20 min before** 1st antibiotic dose.
- Always narrow empirical Rx once organism + susceptibilities known (stewardship).
- **S. aureus bacteremia**: ID consult → **56% ↓ 28-day mortality**.

Infection Control & ID Consult

- **CDC 2007 isolation precaution guidelines** — standard reference.
- Post-exposure prophylaxis: *N. meningitidis*, HIV, *B. anthracis*.
- **ID consult** → ↓ mortality, ↓ LOS, ↓ cost.
- **When to consult ID:**
- **Indications**
  - Difficult-to-diagnose presumed infection.
  - Failure to respond to Rx.
  - Complicated host (transplant, immunosuppressed).
  - Exotic / non-regional disease.

Adjunctive Therapies

- **IVIG** (pooled IgG) — empirical option.
- **Hyperimmune globulin:** CMV, HBV, rabies, vaccinia, tetanus (*C. tetani*), VZV, botulinum toxin (*C. botulinum*).
- IVIG — limited data — used in suspected **staph/strep TSS**.

MNEMONIC

FAGET *Causes of relative bradycardia (pulse-temp dissociation)*
- **F** — Francisella / Q fever (Coxiella)
- **A** — Atypicals — Mycoplasma, Legionella, Leptospira
- **G** — Genus Salmonella (typhoid) + Genus Brucella
- **E** — Ehrlichia / Rickettsia (tick-borne)
- **T** — Typhus + viral hemorrhagic fevers + drugs (β-blocker)

Faget sign = lower HR than expected for the fever. Classic for intracellular organisms.

JOSlerS *Endocarditis skin findings*
- **J** — Janeway lesions — painless, palms/soles
- **O** — Osler nodes — painful, finger pulp
- **S** — Splinter hemorrhages — under nails

Painless = Janeway (palms); Painful = Osler (Ouch).

✎ MCQ CORNER

Q1. 32 y/o returns from Andhra Pradesh with 10 days fever, headache, abdominal pain. Temp 39.5°C, HR 68. Hepatosplenomegaly. Most likely diagnosis?

A. Acute viral hepatitis
✓ **B. Typhoid fever (S. typhi)**
C. Falciparum malaria
D. Dengue hemorrhagic fever

► *Pulse-temperature dissociation (Faget sign) with HSM in returning traveler → enteric fever. S. typhi is the classic cause of relative bradycardia.*

Q2. 55 y/o with stiff neck and fever. CSF: WBC 1500/µL with 90% PMNs, glucose 25 mg/dL, protein 220. Best initial empirical regimen?

A. Ceftriaxone alone
✓ **B. Vancomycin + ceftriaxone + dexamethasone before 1st antibiotic**
C. Meropenem + metronidazole
D. Acyclovir + ceftriaxone

► *Bacterial meningitis profile (PMNs, ↓glucose, ↑protein). Standard empirical = vanco + ceftriaxone. Dexa 0.15 mg/kg given 10–20 min BEFORE 1st abx improves outcome in pneumococcal disease.*

Q3. 40 y/o with chronic headache. CSF shows WBC 800/µL with 60% eosinophils. Best history question?

A. Recent dental work
✓ **B. Travel to Southeast Asia + raw seafood / snail consumption**
C. Pet cat at home
D. Recent tick bite

► *Eosinophilic meningitis (≥50% eos) → think parasites, most commonly Angiostrongylus cantonensis (rat lungworm), acquired via raw seafood, snails, or contaminated produce in Asia/Pacific.*

Q4. ESR is 130 mm/h in a 70 y/o with weight loss and weakness. Which condition is most likely (single highest yield)?

A. Acute viral URI
✓ **B. Subacute bacterial endocarditis, abscess, malignancy, or GCA**
C. Drug fever only
D. Iron deficiency anemia

► *ESR >100 mm/h has 90% PPV for serious disease — infections (~35–40%; SBE, abscess, OM, TB), inflammatory (GCA/RA/SLE), or malignancy (myeloma).*

Q5. Patient with sepsis. Blood cultures pending. After cultures drawn, best empirical antibiotic?

✓ **A. Vancomycin + piperacillin-tazobactam**
B. Ceftriaxone alone
C. Ciprofloxacin + metronidazole
D. Linezolid alone

► *Septic shock empirical = vanco (MRSA cover) + broad-spectrum antipseudomonal β-lactam (pip-tazo, imipenem, meropenem, or cefepime).*

Q6. Which empirical antibiotic should NOT be used for pneumonia?

A. Levofloxacin
B. Linezolid
✓ **C. Daptomycin**
D. Vancomycin

► *Daptomycin is inactivated by pulmonary surfactant — never use for pneumonia. Linezolid is the alternative for MRSA pneumonia.*

◷ LAST MINUTE REVISION

► ID = **2nd leading cause of death** globally; ~16% of cancers infection-related.
► Microbiota ~40 trillion; major reservoir = GI tract.
► **Faget sign** = relative bradycardia → intracellular organisms (typhoid, Brucella, Q fever, Legionella, Lepto, Rickettsia).
► **Rectal > oral by 0.4°C; fever = ≥38.3°C** (FUO def).
► **HR ↑ ~10 bpm per 1°C ↑.**
► Average body temp ↓ ~0.03°C/decade → new normal ~36.7°C.
► Lymph node normal <1 cm; **palpable epitrochlear = pathologic.**
► Endocarditis skin: **Janeway (painless, palms)**, **Osler (painful, pulp)**, splinter hemorrhages.
► **Bacterial WBC → PMN + bands; viral → lymph; parasites → eosinophils.**
► **ESR >100 → 90% PPV serious disease** (infect/inflam/malig).
► ESR slow (≤weekly); CRP fast (daily ok).
► **Bacterial CSF**: WBC >1000 PMN, glucose <40, protein >100, OP >300.
► **Eosinophils ≥50% in CSF = parasitic** (Angiostrongylus).
► **TB CSF**: lymph + low glucose 75%; pellicle smear + AFB.
► Gram needs >10^5 bact/mL; PCR fast but no resistance.
► **Septic shock empirical**: vanco + antipseudomonal β-lactam.
► **Meningitis empirical**: vanco + CTX + dexa 10–20 min BEFORE 1st abx.
► **Daptomycin NOT for pneumonia** (surfactant inactivates).
► **ID consult in S. aureus bacteremia → 56% ↓ 28-day mortality.**
► Hyperimmune globulin: CMV, HBV, rabies, vaccinia, tetanus, VZV, botulinum.

◉ UPDATES

◉ **2007 CDC isolation precautions** — current standard.
◉ **SARS-CoV-2 pandemic** — ~7 million deaths; underscores rapid global spread.
◉ Universal PCR + **shotgun metagenomics** increasingly used for unculturable organisms.
◉ AI/chatbots **not yet replacing** ID consultation.

CH 125 · Molecular Microbial Pathogenesis — Entry · Attachme...

Stage-by-stage view of bacterial pathogenesis: entry, attachment, niche establishment, intracellular survival, secretion systems, immune evasion, toxins. Heavy on board-relevant mechanisms.

Stages of Infection

1. **Colonization** — entry into host body.
2. **Infection** — attachment to niche + avoidance of host defenses.
3. **Disease** — microbial factors + host inflammation → tissue damage.
4. **Transmission** — release into environment, infects new host.

- Host inflammatory response contributes substantially to **symptoms + tissue damage**.
- Microbiota modulates every stage of infection.

Entry into Host

Routes & inocula

- **Skin** — disruption of epidermis (trauma, ulcer, fungal) → cellulitis by *S. aureus*, GAS.
- Diabetic foot ulcer → + *P. aeruginosa*, anaerobes.
- **Respiratory** — droplet nuclei **1–5 µm** (TB, flu, COVID) or fomites.
- Sneeze ≈ 40,000 particles; cough ≈ 3000.
- **GI** — ingestion or person-to-person.
- **Inoculum thresholds**
  - *V. cholerae* = 10^8 (food/water spread).
  - *Salmonella Typhimurium* = 10^5.
  - *Shigella* = 10^1–10^2 → **person-to-person spread**.
- **GU** — colonization of urethra/vagina by fecal flora → ascends to bladder/kidney.
- **Hematogenous** seeding of kidney → pyelonephritis.

Preformed toxin ingestion

- ***S. aureus* enterotoxin** — emetic; dairy, meat, eggs, salads. **Heat-stable toxin**.
- ***B. cereus* cereulide** — K^+ ionophore → emesis; rice, starchy food.
- ***C. botulinum* toxin** — blocks ACh in motor neurons → flaccid paralysis, respiratory failure. **Heat-LABILE toxin, heat-stable spores**.

Attachment

Adhesin classes

Table 125-2 — Adhesins → host receptors

Adhesin	Example	Receptor
Type I pili	FimH, UPEC *E. coli*	Terminal mannose (uroplakin)
Type P pili	Pap, UPEC	Galactose disaccharide
Type IV pili (Tfp)	*N. gonorrhoeae*	CD64, CR3, integrins
MSCRAMM	SdrC, *S. aureus*	β-Neurexin
Opa	*N. meningitidis*	CEACAMs
Flagellum	FliC, *P. aeruginosa*	Asialo-GM1
Autotransporter	Invasin, *Y. pseudotb*	β1-Integrins
Autotransporter	Ag85, *M. tb*	Fibronectin

Pili & flagella highlights

- **Pili (fimbriae)** — pilin polymer, GNB > GPB. **Vaccines unsuccessful so far.**
- Type I FimH — intestinal *E. coli* = oligomannose; UPEC = monomannose.
- **Type IV pili (Tfp)** — related to T2SS; mediate adhesion + DNA uptake + surface motility; microcolony formation (Neisseria, *V. cholerae*).
- Flagella → swimming + chemotaxis; related to T3SS.
- Autotransporter = T5SS subdivision (gram-negative). Examples: **BordetellaFHA**, Shigella IcsA.

Host receptors

- **FUT2** — ~20% have nonfunctional alleles → ↑ bacterial, ↓ viral susceptibility.
- **GM1 ganglioside** — receptor for **cholera toxin**, *Pseudomonas* flagella, *C. perfringens* α-toxin.
- **CFTR** — receptor for *S. Typhi* type IV pili → CF carriers have **relative resistance to typhoid** (heterozygote advantage).
- **CEACAM6** ↑ in IBD → adherent-invasive *E. coli* adheres → Crohn association.
- Heparan sulfate PG → chlamydial OmcB, *E. coli* CNF.

Replicative Niche

- **Extracellular at epithelial surface** — Bordetella, Pseudomonas, Vibrio, Clostridium.
- **Intracellular cytosolic** — Shigella, Francisella, Listeria.
- **Intracellular vacuolar** — Salmonella, Legionella.

Biofilms

- Extracellular polymeric matrix (polysaccharide + DNA).
- Form on **burns, prosthetic material, compromised tissue**.
- Block phagocytosis + impair antibody + antibiotic diffusion → chronic infection.
- Key producers: *S. aureus*, *P. aeruginosa*.

Bacterial Secretion Systems

Fig 125-1 — Major bacterial secretion systems (T3SS, T4SS, T5SS, T6SS) in gram-negative pathogens

Table 125-B — Secretion systems (high yield)

System	Function	Examples
T3SS	Needle/syringe → delivers effectors into host cytosol; **related to flagella**	Shigella, Salmonella, Chlamydia, EPEC
T4SS	Effector + DNA delivery (**conjugation**); G+ and G− and archaea	Legionella, Coxiella, H. pylori
T5SS	**Autotransporter** → adhesion	Bordetella FHA, Shigella IcsA
T6SS	Effector delivery to host or competing bacteria	*F. tularensis*, *Burkholderia*
T7SS (ESX-1)	Phagosome lysis	*M. tuberculosis*

Entry into Cells (Trigger vs Zipper)

Fig 125-2 — Trigger vs Zipper mechanisms of bacterial invasion of nonphagocytic cells

- **Trigger mechanism** — T3SS effectors → actin polymerization → membrane ruffles engulf bacterium.
- **Trigger examples**
  - *Salmonella* — stays in vacuole (SCV).
  - *Shigella* — escapes vacuole into cytosol.
  - *Chlamydia* — stays in inclusion vacuole.
- **Zipper mechanism** — no T3SS; receptor clustering → tight engulfment.
- **Zipper examples**
  - *Y. pseudotb* — invasin → β1-integrin → PI3K/Akt.
  - *L. monocytogenes* — internalin → E-cadherin + Met → β-catenin.

Cytosolic Survival & Cell-to-Cell Spread

- Vacuole-escapers → cytosol → **actin-based motility** → spread to neighbor via protrusion.
- Cytosolic / actin-motility pathogens: ***Listeria, Shigella, Rickettsia* (spotted fever group), *Burkholderia pseudomallei***.
- *L. monocytogenes* escapes vacuole via **listeriolysin O** (pore-former) + phospholipases.
- *Burkholderia* → induces giant multinucleated cell formation (T6SS).

Vacuolar Survival

- Block phagosome–lysosome fusion + endosomal maturation.
- **Legionella-containing vacuole (LCV)** — T4SS effectors; LCV resembles ER; >300 effector proteins.
- **Coxiella-containing vacuole (CCV)** — T4SS; lysosome-like, acidic pH activates effectors.
- **Salmonella** — SCV, two distinct T3SSs (invasion vs maintenance).
- ***F. tularensis*** — T6SS → escapes phagosome, replicates in macrophage cytosol.
- ***M. tuberculosis*** — **ESX-1 T7SS** lyses phagosome.

Innate Immune Evasion — Complement

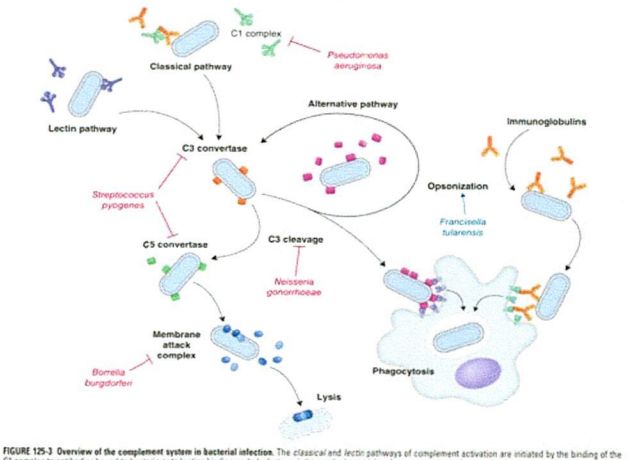


FIGURE 125-3 Overview of the complement system in bacterial infection. The *classical* and *lectin* pathways of complement activation are initiated by the binding of the C1 complex to antibodies bound to bacteria or to lectins binding carbohydrate moieties on the bacterial surface, respectively. A cascade of proteolytic cleavage generates the C3 convertase complex on the bacterial surface, which, upon activation, leads to C5 convertase formation. In the alternative pathway, spontaneous C3 cleavage forms alternative C3 products that lead to bacterial opsonization and to amplification of C3 convertase activation. The three complement pathways converge at the point of C3 convertase activation. In a manner similar to that seen for bacteria opsonized with circulating immunoglobulin, opsonized bacteria bind to surface receptors of phagocytes, triggering their engulfment for destruction and stimulation of the adaptive immune system. The C5 convertase, generated by conventional C3 convertase activation, recruits

Fig 125-3 — Complement system in bacterial infection: classical, lectin, and alternative pathways → MAC

- **Classical pathway** — Ab–C1 binding.
- **Lectin pathway** — lectin–carbohydrate.
- **Alternative pathway** — spontaneous C3 cleavage.
- All converge → **C3 convertase → C5 convertase → MAC.**
- **Strep evasion**: ScpA/SpeB proteases degrade C; M protein binds C4BP; hyaluronic capsule shields.
- **Borrelia** → CD59-like protein → blocks MAC.
- **Gram-positives** resist MAC via thick peptidoglycan.
- *Francisella*, *Yersinia* → **promote** opsonization to enter phagocyte.

Lysosomes, AMPs & Oxidative Burst

- **Lysozyme** hydrolyzes peptidoglycan glycan chains → effective vs **gram-positives**; GNB peptidoglycan protected between membranes.

- **Defensins, cathelicidins** — cationic AMPs target anionic LPS / teichoic acid; bacteria modify surface charge to evade.
- **NADPH oxidase** → superoxide burst in neutrophil phagosome.
- **Chronic granulomatous disease (CGD)** = nonfunctional NADPH oxidase → recurrent bacterial + fungal infections.
- Bacterial defenses: SOD, peroxiredoxin, catalase.

NETs, PRRs & Pyroptosis

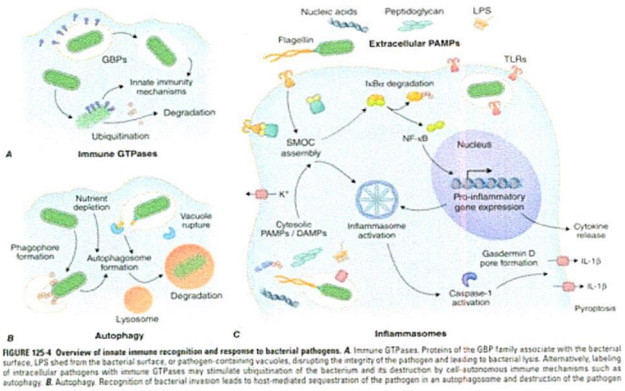


FIGURE 125-4 Overview of innate immune recognition and response to bacterial pathogens. *A*, Immune GTPases. Proteins of the GBP family associate with the bacterial surface, LPS shed from the bacterial surface, or pathogen-containing vacuoles, disrupting the integrity of the pathogen and leading to bacterial lysis. Alternatively, labeling of intracellular pathogens with immune GTPases may stimulate ubiquitination of the bacterium and its destruction by cell-autonomous immune mechanisms such as autophagy. *B*, Autophagy. Recognition of bacterial invasion leads to host-mediated sequestration of the pathogen in an autophagosome and destruction of the pathogen

Fig 125-4 — Innate immune recognition: TLRs, GBPs, autophagy, inflammasome → pyroptosis

- **NETs** = neutrophil extracellular traps — decondensed chromatin mesh.
- ***S. aureus* nuclease** degrades NETs → deoxyadenosine = macrophage toxin.
- Strep DNases degrade NETs too.

Table 125-3 — PRRs & ligands

PRR	Ligand
TLR2 (+TLR1 or TLR6)	Lipoproteins
TLR4	**LPS**
TLR5	**Flagellin**
TLR9	**CpG DNA** (unmethylated)
NLRP1	Enzymatic cleavage
NLRP3	Ionic flux, mitochondrial damage
NLRP6	Lipoteichoic acid, RNA
NAIP/NLRC4	Flagellin / T3SS
STING	Cyclic dinucleotides
NOD1/NOD2	Peptidoglycan

♥ NEET SS HIGHLIGHTS

- ♥ **NOD2 mutation → Crohn disease.**
- ♥ **TLR4 = LPS receptor** (gram-negative sepsis).
- ♥ **TLR9 = unmethylated CpG DNA.**
- ♥ **cGAS-STING** senses cytosolic DNA → type I IFN.
- ♥ **Inflammasome (NLRP3)** → caspase-1 → GSDMD pore + IL-1β/IL-18 → **pyroptosis.**
- ♥ **CGD** = NADPH oxidase defect → catalase-positive infections.

- **PAMP masking:**
- **Examples**
  - *Y. pestis* → tetra-acylated LPS in human host (vs hexa- in flea) → no TLR4.
  - Tetra-acylated LPS: *F. tularensis*, *H. pylori*, *P. gingivalis*.
  - *H. pylori* flagellin epitope evades TLR5.
  - GAS Sda1 DNase degrades CpG DNA.
- NF-κB inhibition by Shigella, Chlamydia, Burkholderia, Salmonella effectors (E3 ligases, deubiquitinases, MTases).
- ***B. anthracis* lethal factor** = metalloprotease, cleaves MAPK.

Adaptive Immune Evasion

- **M. tuberculosis** latent infection — induces IL-6, IL-10, TGF-β → ↓ IFN-γ + MHC-II.
- **B. pertussis** → dendritic IL-10 → drives regulatory T cells.
- **Salmonella SteD** (T3SS) → MARCH8 ubiquitinates MHC-II → ↓ surface expression.
- **Yersinia YopH** (Tyr phosphatase) → dephos B-/T-cell receptors.
- ***S. aureus* superantigens (TSST-1)** → bind invariant MHC + TCR → polyclonal T-cell activation → **cytokine storm → TSS.**

❦ CLINICAL PEARLS

- ❦ **SCID** = T+/−B cell defect → severe bacterial, viral, fungal infections.
- ❦ **X-linked agammaglobulinemia** → ↓ Ab → recurrent extracellular bacterial infections.
- ❦ **CGD** (NADPH oxidase) → catalase + organisms (*S. aureus*, *Burkholderia*, *Serratia*, *Nocardia*, fungi).
- ❦ **Latent TB** = immune control without elimination; lifetime reactivation risk ~5–10%.

Bacterial Cytotoxins

AB toxins

- **A subunit** = enzymatic activity; **B subunit** = receptor binding.
- **Shiga toxin** (Shigella, EHEC) — inhibits 28S rRNA → HUS.
- **Diphtheria toxin** (*C. diphtheriae*) — ADP-ribosylates EF-2 → halts protein synthesis.
- **Pertussis toxin** — ADP-ribosylates Gαi → ↑ cAMP.
- **Cholera toxin (CTX)** — ADP-ribosylates Gαs → ↑↑ cAMP → CFTR opens → Cl^- secretion → **massive watery diarrhea.**
- **Pseudomonas exotoxin A** — ADP-ribosylates EF-2 (like diphtheria).

Pore-forming toxins

- Examples: **pneumolysin** (S. pneumo), **perfringolysin O** (C. perfringens), **anthrax toxin**, **listeriolysin O.**
- ***S. aureus***: leukocidins (PVL), α/γ-hemolysins, phenol-soluble modulins.
- Specificity driven by surface receptor.
- Defenses: **exosomes act as decoys** for cytolysins.

Other toxins

- *C. perfringens* + *P. aeruginosa* non-AB toxins → ADP-ribosylate EF-2.
- ***C. difficile*** toxins → target Rho GTPase → cytoskeletal disruption + **pyrin inflammasome activation.**
- *V. cholerae* MARTX → actin crosslinking → barrier failure.

Tissue Damage, Transmission & Phages

- Tight junction disruption → barrier failure → invasion (C. perfringens, V. cholerae, EHEC, Shigella, S. Typhi).
- SPATEs (Shigella, E. coli) cleave junctions + mucins.

- Transmission aligned with entry: cough → respiratory; diarrhea → fecal-oral.
- **Cholera seasonality** linked to bacteriophage blooms in aquatic reservoir.

✦ MNEMONIC

SAFE-Coke *AB toxins → ADP-ribosylation targets*

S — Shiga / Shiga-like — 28S rRNA (60S)
A — ADP-ribosylates EF-2 → Diphtheria, Pseudomonas exotoxin A
F — Forskolin-like ↑ cAMP — Pertussis (Gαi), Cholera (Gαs)
E — Edema factor (anthrax) — adenylate cyclase → ↑ cAMP
Co — C. botulinum — blocks ACh release
k — Tetanospasmin — blocks GABA/glycine release
e — Heat-stable: S. aureus enterotoxin, B. cereus emetic toxin

Cholera ↑ Gαs; Pertussis ↓ Gαi (both → ↑ cAMP).

TZ-Jets *Trigger vs Zipper — Invasion mechanisms*

T — Trigger → T3SS → Salmonella, Shigella, Chlamydia
Z — Zipper → receptor cluster → Yersinia (invasin), Listeria (internalin)

Trigger = pushed in (ruffles). Zipper = pulled in (close apposition).

✎ MCQ CORNER

Q1. Which pathogen escapes the phagosome into the cytosol using listeriolysin O?

A. Salmonella Typhimurium
✓ **B. Listeria monocytogenes**
C. Mycobacterium tuberculosis
D. Legionella pneumophila

► *Listeria escapes via listeriolysin O (pore-former) + phospholipases, then uses actin-based motility (ActA) to spread cell-to-cell.*

Q2. A patient develops massive watery diarrhea. Cholera toxin acts via which mechanism?

A. ADP-ribosylation of EF-2
✓ **B. ADP-ribosylation of Gαs → ↑ cAMP → CFTR Cl^- secretion**
C. Cleavage of 28S rRNA
D. Inactivation of Rho GTPase

► *CTX ADP-ribosylates Gαs (locks adenylate cyclase ON) → ↑↑ cAMP → activates CFTR → massive Cl^- and water secretion.*

Q3. A 6 y/o with recurrent S. aureus skin abscesses, pulmonary aspergillosis. Defect?

A. B-cell deficiency (XLA)
B. Complement deficiency
✓ **C. NADPH oxidase deficiency (CGD)**
D. Toll-like receptor 4 mutation

► *Chronic granulomatous disease — NADPH oxidase defect → cannot kill catalase-positive organisms (S. aureus, Burkholderia, Aspergillus, Nocardia, Serratia).*

Q4. Cytosolic DNA sensor that triggers type I interferon via STING?

A. TLR9
✓ **B. cGAS**
C. NLRP3
D. NOD2

► *cGAS detects cytosolic DNA → produces cGAMP → activates STING → IRF3/NF-κB → type I IFN. TLR9 senses CpG DNA in endosomes (not cytosol).*

Q5. Toxic shock syndrome from S. aureus is caused by:

A. Pore-forming hemolysin lysing erythrocytes
✓ **B. Superantigen (TSST-1) cross-linking invariant MHC-II and TCR → cytokine storm**
C. ADP-ribosylation of EF-2
D. Direct invasion of endothelium

► *Superantigens bypass normal antigen processing and bind invariable regions of MHC-II and TCR → polyclonal T-cell activation (up to 20%) → massive cytokine release → shock.*

Q6. Which secretion system delivers DNA via conjugation as well as toxin effectors?

A. Type II
B. Type III
✓ **C. Type IV**
D. Type VI

► *T4SS — most diverse; mediates conjugation (plasmid DNA transfer) AND effector delivery (Legionella, Coxiella, H. pylori CagA).*

LAST MINUTE REVISION

► **Trigger** (T3SS-driven ruffles) = Salmonella, Shigella, Chlamydia. **Zipper** = Yersinia, Listeria.
► **Cytosolic** = Listeria, Shigella, Rickettsia, Burkholderia. **Vacuolar** = Salmonella, Legionella, Coxiella.
► **Listeriolysin O** → Listeria vacuole escape; **ESX-1 T7SS** → TB.
► **T3SS** = needle/syringe, related to flagella. **T4SS** = conjugation + effectors (Legionella).
► **Cholera toxin** → Gαs ADP-ribose → ↑ cAMP → CFTR.
► **Pertussis toxin** → Gαi ADP-ribose → ↑ cAMP.
► **Diphtheria + Pseudomonas exo A** → ADP-ribose EF-2 → halt translation.
► **Shiga toxin** → cleaves 28S rRNA → HUS.
► **Anthrax lethal factor** → cleaves MAPK; **edema factor** → calmodulin-AC → ↑ cAMP.
► **Botulinum** blocks ACh release (flaccid); **Tetanus** blocks GABA/glycine (spastic).
► **Heat-stable**: S. aureus enterotoxin, B. cereus cereulide, E. coli ST, V. cholerae.
► **Superantigens** (TSST-1) cross-link invariant MHC-II + TCR → cytokine storm.
► **NOD2** → Crohn; **TLR4** = LPS; **TLR5** = flagellin; **TLR9** = CpG.
► **NLRP3 inflammasome** → caspase-1 → IL-1β + GSDMD pore → pyroptosis.
► **CGD** = NADPH oxidase defect → catalase-positive infections (S. aureus, Burkholderia, Aspergillus, Nocardia, Serratia).
► **Biofilms** (S. aureus, P. aeruginosa) — block phagocytosis + antibiotic diffusion.
► **CF/CFTR** = S. Typhi receptor → CF carriers resistant to typhoid.
► **FUT2 non-secretor** ~20% → ↓ viral, ↑ bacterial susceptibility.
► Cholera inoculum 10^8 (water); Shigella 10^1–10^2 (person-to-person).
► **Y. pestis** tetra-acylated LPS in human → escapes TLR4.

UPDATES

◉ **cGAS-STING** pathway — major focus for adjuvant + antiviral development.
◉ **GSDMD pore** + **pyroptosis** — emerging therapeutic target in sepsis.
◉ **Innate immune training** (epigenetic priming) — mechanism behind BCG heterologous protection.

CH 126 · Microbial Genomics & ID — NAATs · Resistance · Geno...

Genomic tools in ID: PCR/NAAT, 16S, metagenomics, sequence-based resistance, host transcriptomics, genomic epidemiology (cholera, Ebola, SARS-CoV-2), vaccine design (reverse vaccinology, mRNA).

Goals & Glossary

- Clinical micro lab goals: **presence + ID + susceptibility + virulence factors**.
- Genomic shift: from growth/biochem → **nucleic acid-based** → faster, more sensitive, broader.

Table 126-1 — Key terms

Term	Definition
Contig	Continuous DNA fragment from overlapping reads (de novo assembly)
HGT	Horizontal gene transfer — transformation, conjugation, transduction
Metagenomics	DNA from **multiple species directly from sample**, no culture
MLST	Multilocus sequence typing — prespecified gene set
NGS	Next-generation sequencing — millions of parallel reads
NAAT	Nucleic acid amplification (PCR, LCR)
SNP	Single nucleotide polymorphism = genetic distance measure
Transcriptome	Full mRNA catalog (microarray, RNA-Seq)
WGS	Whole genome sequencing
Microbial GWAS	Genotype ↔ phenotype (resistance, virulence)

Limitations of Traditional Diagnostics

- **Serology** = retrospective (acute + convalescent); cannot distinguish active vs latent.
- **TB**: PPD insensitive in active disease + vaccine cross-reactivity; IGRAs limited in immunocompromised. Neither distinguishes active vs latent.
- **Lyme**: IgG = past exposure in endemic areas; IgM early disease sens 50%, spec 80%.
- **Syphilis**: needs both **non-treponemal** (RPR) + **treponemal** (FTA-ABS) tests.
- **Antigen tests** validated for limited pathogens: HBsAg, HIV p24, SARS spike, S. pneumo, Legionella ser-1, Crypto, Histo, galactomannan (Aspergillus), β-glucan.

Nucleic Acid Diagnostics (NAAT/PCR)

- **Viral NAATs** = standard care: HIV, HCV, HBV, flu, SARS-CoV-2, RSV.
- **HIV PCR** sensitivity → 20 copies/mL → genotypic resistance routine before ART.
- **Fastidious bacteria NAATs**: *N. gonorrhoeae*, *C. trachomatis*, *Ehrlichia*, *Anaplasma*.
- **C. difficile NAAT** — detects toxins A/B + NAP1 (hypervirulent) marker.
- **Diagnostic escape** — Swedish *C. trachomatis* deletion missed by commercial NAAT.
- **MALDI-TOF mass spec** → rapid ID from positive blood culture (supplants biochem).
- **16S rRNA PCR** = most conserved bacterial gene → broad bacterial ID for culture-negative cases (e.g., *M. chimaera* post-cardiac surgery).
- **Syndromic panels** (multiplex PCR): respiratory virus panel, GI panel, meningoencephalitis panel.
- **CRISPR-based diagnostics** — Cas12a/Cas13a + isothermal preamp → field-deployable, lyophilized.

Metagenomic Pathogen Discovery

- **Shotgun metagenomics** = unbiased sequencing of all DNA/RNA in sample.
- Workflow: extract → sequence → align to host (subtract) → align to known microbes → **de novo assemble unmapped → contigs → phylogeny**.
- **SARS-CoV-2**: identified via metagenomics within ~**1 month** of first case; full genome in 5 days; NAAT next day.
- Compare with SARS-CoV (2003): full genome ~6 months post-recognition.
- Cell-free DNA sequencing of plasma — FDA-cleared; identifies pathogens when culture fails.

CLINICAL PEARLS

- **Cord colitis** — new *Bradyrhizobium* species discovered by metagenomics.
- **2011 German diarrheal outbreak** — *E. coli* O104:H4 identified via metagenomics.
- **Neuroleptospirosis** in immunocompromised — diagnosed via CSF metagenomics; treated with penicillin G.
- **Modern Koch's postulates** — sequencing identifies candidates; experimentation needed for causality.

Sequence-Based Resistance

Table 126-2 — Resistance genes by PCR

Pathogen	Gene	Drug Resistance
MRSA	**mecA** (PBP2A)	Methicillin / oxacillin
VRE	**vanA / vanB**	Vancomycin
MDR-TB	**rpoB** (95%)	Rifampin → flag for MDR
MDR-TB INH	**inhA + katG** (~85%)	Isoniazid
CRE	**KPC, NDM, OXA-48, IMP, VIM**	Carbapenems
HIV	RT, protease, integrase mutations	ARV resistance

- **HIV K103N** → predicts **efavirenz** (NNRTI) resistance.
- **Xpert MTB/RIF** — single PCR detects TB + rifampin resistance directly from sputum → hours not weeks; transformative in

Africa/Asia.

- **FQ resistance** = 5 mechanisms: ↓ import, ↑ efflux, target mutation, drug modification, target shielding → combinatorial → genotyping limited.
- **5% of MSSA** carry nonfunctional *mecA* → genotypic false-positive (would mis-treat with vancomycin).
- **mcr-1** = plasmid-borne **colistin resistance** (China 2015) → transferable last-resort resistance.
- **WGS for TB susceptibility** — UK routinely uses parallel with phenotypic AST.

Host-Based Diagnostics

- **Host transcriptional signature** distinguishes active vs latent TB (~400 transcripts; refined to 3-transcript).
- Distinguishes bacterial vs viral URI; even gram-positive vs gram-negative.
- Procalcitonin = limited (only narrow applications).
- **Genetic susceptibility**:
- **Examples**
  - **Duffy-negative** → resistance to *P. vivax*.
  - **HbAS** heterozygote → protection vs falciparum malaria.
  - **CCR5-Δ32** homozygous → HIV resistance.
  - **LTA4H** polymorphism → modulates steroid benefit in TB meningitis.
  - Severe COVID-19 associated with type I IFN autoantibodies + specific loci.

Therapeutics: Genomics in Drug & Vaccine Design

Reverse vaccinology

- Sequence → identify conserved surface antigens → express → test immunogenicity.
- Useful for **unculturable / poorly immunogenic** pathogens.
- **SARS-CoV-2 mRNA vaccines** — design started **day after** genome shared; fastest vaccine in history.

Vaccine impact on populations

- **PCV-7 → serotype replacement**: nonvaccine pneumococcal serotypes rose; PCV-13 broader cover.
- Some pneumococci recombine vaccine-target capsule with non-vaccine cassette → escape.
- **HPV vaccines** target highest-risk types 16, 18 (cervical Ca).
- **RTS,S/AS01 malaria vaccine** — ↓ efficacy against parasites with circumsporozoite mutations.
- **Seasonal flu**: genomic + antigenic cartography → annual strain selection; Southeast Asia = origin of new diversity.

Antibiotic discovery

- Genome-scale essential-gene ID → therapeutic targets.
- Transcriptional signatures distinguish drug class: β-lactams vs FQ vs aminoglycosides.
- **Anti-virulence drugs** — emerging concept (block virulence, not kill).

Genomic Epidemiology

- **WGS resolves outbreaks** at SNP level — better than MLST or PFGE.
- **PulseNet** (CDC) now uses WGS for foodborne pathogen tracking.
- **Nextstrain.org** — open real-time genomic epi.
- **Wastewater surveillance** — SARS-CoV-2 "nowcasting"; CDC building national network.

CLINICAL PEARLS

- **Cholera in Haiti 2010** — WGS showed strain from **Nepal** (UN peacekeepers), not Latin America.
- **HIV-1 Group M** dated to **1920s Kinshasa** (Belgian Congo) via molecular clock.
- **Ebola West Africa 2013–16** — phylogeography traced spread (Liberia, Sierra Leone, Guinea).
- **SARS-CoV-2** — MRCA late Nov/early Dec 2019, Wuhan. **D614G** spike mutation displaced founder strain.
- **Omicron** first identified **South Africa** → enabled global preparation.
- **Burkholderia pseudomallei** contamination in aromatherapy spray — outbreak solved via WGS.
- **Pseudomonas in eyedrops, Exserohilum in epidural steroids, Fusarium in epidural anesthetics, E. coli O157:H7 in beef, M. chimaera in cardiac bypass heater-coolers** — all WGS-resolved.

Epidemic Potential & Pathogen Evolution

- R_0 (basic reproduction number) = 2° infections per 1° case.
- R_0 >1 → epidemic potential; R_0 <1 → extinction.
- **2009 H1N1 R_0** estimated at **1.2** via genomic analysis (epi data 1.4–1.6).
- **Influenza segmented genome** → reassortment in coinfected cells (2009 H1N1 = avian+swine+human).
- **H5N1 transmissibility** — 5 lab mutations needed for ferret-to-ferret spread.
- Listeria — distinct clonal complexes have CNS tropism vs neonatal/maternal — molecular virulence linked to clinical phenotype.

COVID-19 Genomic Milestones

- **~1 month**: pathogen ID via metagenomics.
- **5 days post-ID**: full genome assembled.
- **+1 day**: NAAT (RT-qPCR) released.
- **+1 day after sequence share**: mRNA vaccine design began (Spike target informed by SARS/MERS homology).
- Major variants timeline: **founder → D614G (early 2020) → Alpha → Delta → Omicron + subvariants**.

NEET SS HIGHLIGHTS

- **16S rRNA** = universal bacterial ID gene (most conserved).
- **mecA** = MRSA marker; **vanA/vanB** = VRE; **rpoB** = TB ID + rifampin resistance.
- **Xpert MTB/RIF** = single PCR for TB + rifampin resistance in hours.
- **HIV K103N** → efavirenz resistance.
- **D614G** = first major SARS-CoV-2 spike variant.
- **R_0 >1** = epidemic; **HIV-1 Group M** origin Kinshasa 1920s.
- **PCV serotype replacement** — non-vaccine serotypes rise after vaccination.
- **CRISPR Cas12a/Cas13a** = field-deployable detection.

✦ MNEMONIC

META-COVID *Genomic milestones of SARS-CoV-2*

M — Metagenomic ID — ~1 month
E — Encoding (full genome) — 5 days later
T — Test (RT-qPCR NAAT) — +1 day
A — Antigen vaccine design — Day after sequence share
C — Clinical homology to SARS/MERS → Spike target
O — Omicron — first in South Africa
V — Variants tracked in real time on nextstrain
I — Immune evasion variants — Alpha → Delta → Omicron
D — D614G — first major spike variant (early 2020)

Fastest pandemic genomic response in history.

16S-mec-van-rpoB *Key bacterial PCR targets*

16S — rRNA — broad bacterial species ID
mecA — MRSA (PBP2A)
vanA/B — VRE
rpoB — TB species + rifampin resistance

✎ MCQ CORNER

Q1. A 40 y/o with culture-negative endocarditis on echo. Best next test for organism identification?

A. Repeat blood cultures off antibiotics
✓ **B. Cell-free DNA metagenomic sequencing of plasma + 16S PCR of excised valve**
C. ELISA for Bartonella IgG only
D. Empirical 6 weeks of ceftriaxone

► *Culture-negative endocarditis (Bartonella, Coxiella, HACEK, fungi) — molecular diagnostics (cf-DNA, 16S PCR) markedly improve identification. Serology helps but molecular is broader.*

Q2. Xpert MTB/RIF detects rifampin resistance via mutations in which gene?

A. katG
✓ **B. rpoB**
C. inhA
D. embB

► *rpoB encodes RNA polymerase β-subunit, target of rifampin; ~95% of resistance maps to a short region. katG/inhA are for INH; embB for ethambutol.*

Q3. Which mutation predicts efavirenz resistance in HIV?

A. M184V in reverse transcriptase
✓ **B. K103N in reverse transcriptase**
C. L90M in protease
D. N155H in integrase

► *K103N → NNRTI resistance (efavirenz, nevirapine). M184V → 3TC/FTC; L90M → PI; N155H → INSTI.*

Q4. The Haitian cholera outbreak (2010) was traced via WGS to which source?

A. Endemic Latin American Vibrio strain
B. Environmental contamination of local rivers
✓ **C. Strain imported from South Asia (Nepal) via UN peacekeepers**
D. Recombinant strain from Caribbean shellfish

► *Phylogenetic analysis showed the Haitian V. cholerae was nearly identical to South Asian (Nepali) strains, supporting human-mediated import.*

Q5. Which is the most likely cause of "diagnostic escape" in molecular testing?

A. Bacterial overgrowth in transport media
✓ **B. Pathogen acquires deletion/mutation in PCR primer-binding site**
C. Improper sample collection
D. Loss of antibiotic susceptibility data

► *Swedish C. trachomatis variant with a deletion in the commercial NAAT target site evaded detection → spread silently. Highlights need to monitor molecular assays.*

◷ LAST MINUTE REVISION

► **16S rRNA PCR** → broad bacterial ID (especially culture-negative cases).
► **mecA** = MRSA; **vanA/vanB** = VRE; **rpoB** = TB + rifampin resistance.
► **Xpert MTB/RIF** = single test for TB + rifampin resistance in hours.
► **HIV K103N** → efavirenz resistance.
► **5% of MSSA** carry nonfunctional mecA → false-positive genotypic MRSA.
► **mcr-1** = plasmid colistin resistance (China 2015) — transferable last-resort resistance.
► **Metagenomic shotgun** → unbiased pathogen discovery (cord colitis, neuroleptospirosis, SARS-CoV-2).
► **Modern Koch postulates** + experimental validation needed for causality.
► **CRISPR Cas12a/Cas13a** diagnostics — field-deployable, lyophilized.
► **Reverse vaccinology** → mRNA vaccines (SARS-CoV-2 designed in 1 day post genome share).
► **Serotype replacement** after PCV-7 → development of PCV-13 (& PCV-15/20).
► **RTS,S malaria vaccine** — circumsporozoite mutations reduce efficacy.
► **2009 H1N1** = reassortment of avian + swine + human strains; R_0 ~1.2.
► **Cholera Haiti** = imported Nepal strain (WGS).
► **HIV-1 Group M** origin: Kinshasa, 1920s.
► **Omicron** first identified in South Africa.
► **D614G** = first major SARS-CoV-2 spike variant.
► **LTA4H** polymorphism → modulates steroid benefit in TB meningitis (personalized).
► **Wastewater surveillance** = population-level pathogen monitoring (SARS-CoV-2, polio).
► **nextstrain.org** = real-time open genomic epi platform.

◉ UPDATES

◉ **mRNA vaccine platform** validated by SARS-CoV-2 — now extended to RSV, flu, malaria, HIV trials.
◉ **CRISPR-Cas13 (SHERLOCK) + Cas12 (DETECTR)** — field-deployable molecular diagnostics.
◉ **FDA-cleared cf-DNA metagenomic test** (Karius) for cryptic systemic infection.
◉ **Wastewater surveillance** — CDC building national network post-COVID.
◉ **Whole-genome AST for TB** — UK routine.
◉ **Long-read sequencing** (Nanopore, PacBio) — improving plasmid + mobile element resolution.