V cholerae virulence

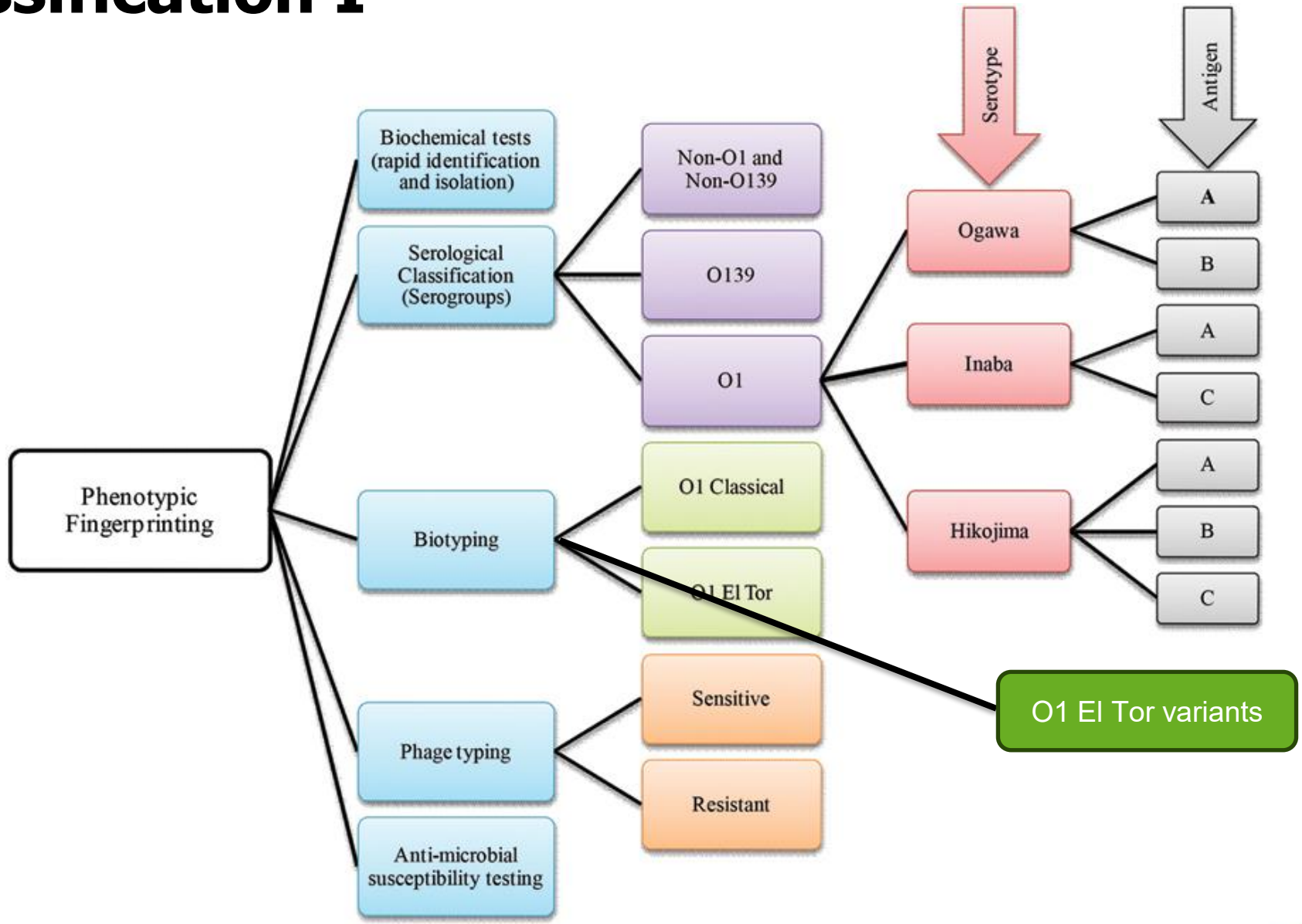
Intended Learning Objectives

- An overview of *V cholerae* virulence
- Understanding the output from *V cholerae* genomic analysis

Outline

1. Classification
2. Main genes and pathogenicity islands
3. The cholera toxin
4. Resistance in *V cholerae*
5. Vaccines

Classification I

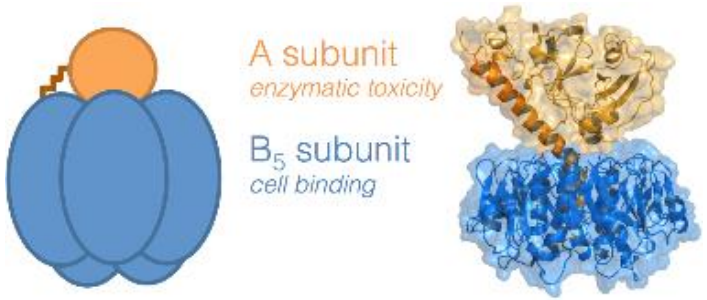


Classification I

- Serogroups based on the O antigen of LPS (N>200)
  Serogroups O1 and O139 associated with epidemics:
  - O1:
    - O1 serotypes (antigens): Ogawa, Inaba, Hikojima
    - O1 biotypes (phenotypic and genetic markers):
      - ctxB1 → **Classical**
      - ctxB3 → **El Tor** — 60s
        - More efficient at host-to-host transmission
        - Better survival and fitness
        - Higher occurrence of asymptomatic than symptomatic cases
      - ctxB7 → **El Tor variants** — 90s
        - El Tor genetics, but hybrid classical/El Tor phenotypes
        - Became the dominant 7PET
        - Secrete higher amounts of cholera toxin than the original El Tor
  - O139: hasn't replaced O1, uncommon since the early 2000s. — 90s
  - Non-O1 non-O139: not cholera disease

Main virulence genes I

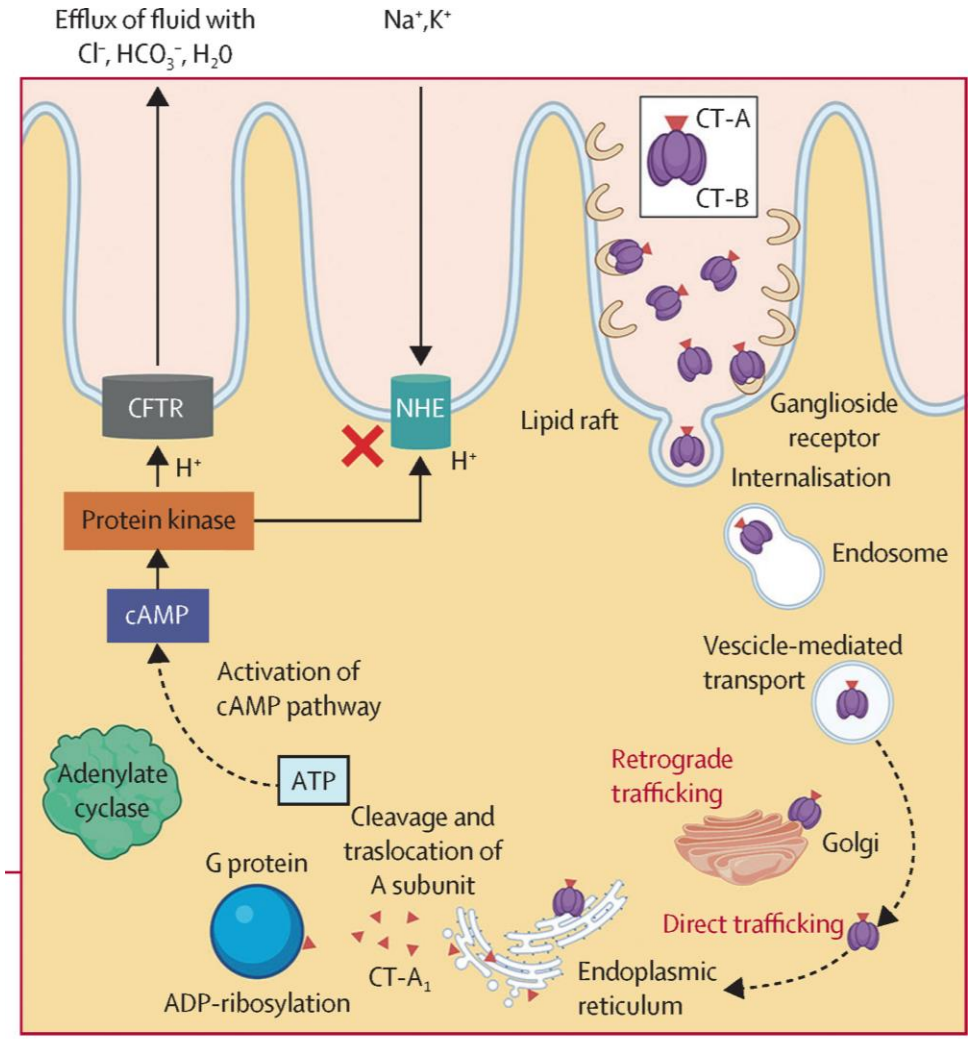
- **Cholera Toxin (CT):**
  - Subunit CTA (encoded by *ctxA*): enzymatic activity
  - Subunit CTB (encoded by *ctxB*): pentameric, binding and endocytosis
  - On the filamentous CTXΦ phage, which is integrated on the *dif* sites of either or both chromosomes.
  - Disrupts ion transport in intestinal cells, leading to profuse watery diarrhea.



Van Kessel and Camilli 2024 https://doi.org/10.1128/jb.00248-24

Inside the epithelial cell

1. CTB attaches to membrane by binding to the GM1 ganglioside receptor on small intestine epithelium
2. Endocytosis of the holotoxin
3. CTA and CTB dissociate
4. CTA activates G proteins → ↑ adenylate cyclase activity → ↑ intracellular cAMP → activation of protein kinase A → inhibition of absorption of Na^+ and K^+ + opens the CFTR channel → loss of Cl^-, $HCO3^-$, and water
5. Role of the gut microbiome: genera e.g. *Prevotella* and *Bifidobacterium* have been associated to inhibition of virulence gene expression



Kanungo et al. 2022 https://doi.org/10.1016/S0140-6736(22)00330-0

Main virulence genes II

- **Toxin-coregulated pilus (TCP):**
  - Encoded in VPI-1 (*tcpA-F*)
  - Major intestinal colonisation factor and the receptor for CTXΦ

- **ToxR / ToxS system:**
  - Regulates expression of cholera toxin, TCP and other virulence factors.

Kanungo et al 2022 https://doi.org/10.1016/S0140-6736(22)00330-0
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Genomic islands - definition

- **Large DNA segments in a genome that have been acquired through HGT**
- **Usual characteristics:**
  - Show different GC content compared to the host genome
  - Flanking repeat sequences
  - Commonly inserted at tRNA genes or flanked by mobile genetic elements like integrases or transposases.
- **Function:** Can carry clusters of genes that provide adaptive advantages (e.g., antibiotic resistance, virulence factors, metabolic pathways).
- **Examples:** Pathogenicity islands, resistance islands

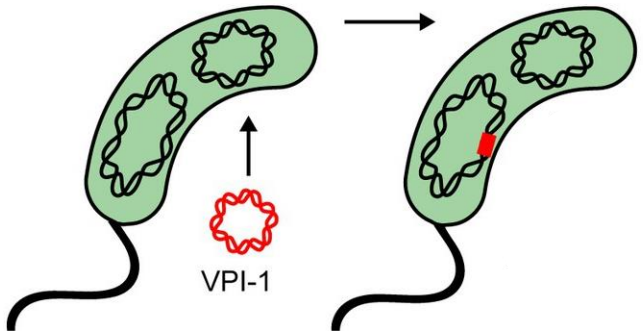


Image adapted from Montero et al. 2023. https://doi.org/10.3389/fmed.2023.1155751

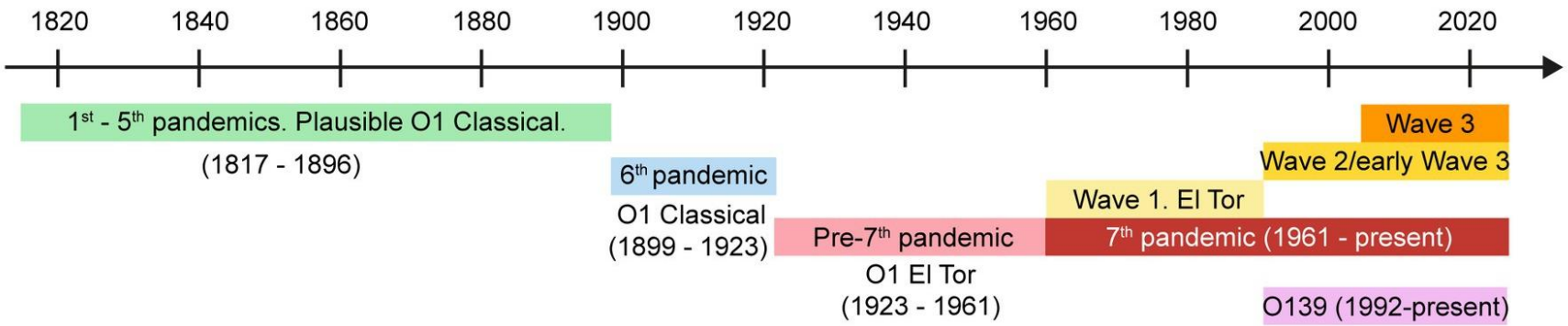
Genomic islands

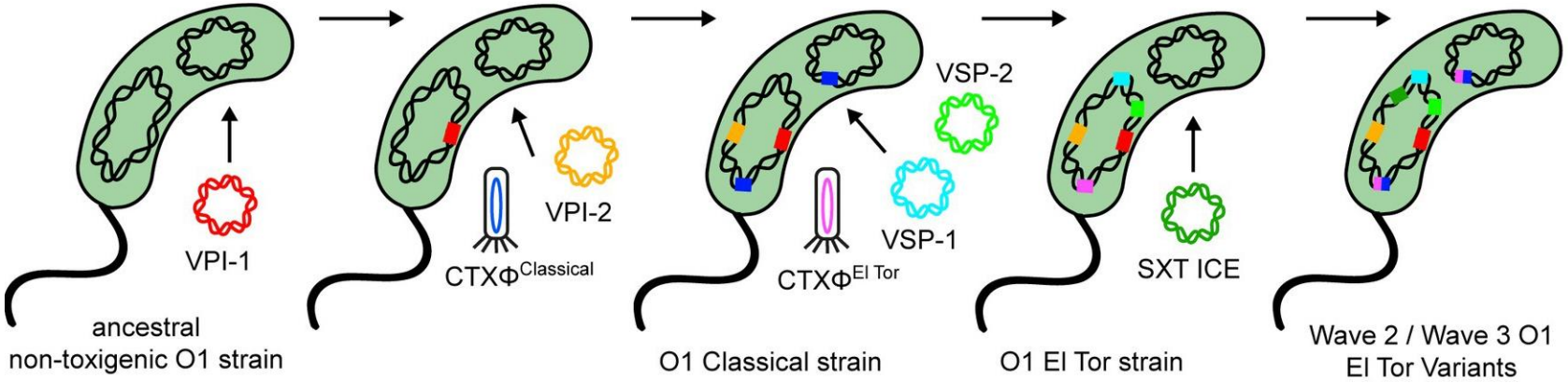
Almost all the 7th pandemic *V cholerae* strains carry these four:

- **Vibrio pathogenicity island-1 (VPI-1)** ~41.3 kb **:**
  - Encodes 31 genes, incl. TCP, ToxT, TcpA, TcpP, TcpH, and accessory colonization factors (ACFs).
  - Includes an integrase (intV) and transposase (vpiT), facilitating mobility and integration.
  - Crucial for colonization and acquisition of CTXΦ
- **Vibrio pathogenicity island-2 (VPI-2)** ~57 kb **:**
  - Associated with sialidase and neuraminidase production, which expose GM1, promoting CTB binding, aiding in mucosal invasion. It can excise.
- **Vibrio seventh pandemic island1 & 2 (VSP-1 & VSP-2)** ~16 kb & ~26.9 kb **:**
  - Characteristic of 7th pandemic El Tor lineages (7PET)
  - Anti-phage defense roles, helps pandemic strains persist in phage-rich aquatic reservoirs
  - Enhance environmental fitness and survival of pandemic strains
- **Integrative conjugative elements (ICE):**
  - SXT ICE: carries multiple AMR genes. Transfer induced by the SOS response triggered by antibiotics.
  - ICEVchHai1: SXT ICE variant in the Haiti epidemic strain

O'Hara et al 2022 https://doi.org/10.1371/journal.pgen.1010250

Evolutionary acquisition of virulence





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Acquisition of SXT ICE likely influenced the population shift from the Wave 1 to Wave 2/3 strains

Resistance

- Cholera is managed by rehydration (intravenous, oral if only some/no dehydration)
- Antibiotics indicated for severe cases, pregnant women, acute malnutrition, or chronic conditions:
  - Historically: streptomycin, chloramphenicol, tetracycline, sulphamethoxazole-trimethoprim
  - First line: doxycycline, azithromicin, ciprofloxacin
- AMR associated with:
  - During the 60s, mainly due to **mutations** of chromosomal regions
  - MDR today is facilitated by acquisition of **mobile genetic elements**:
    - Plasmids of incompatibility type C (***IncC***)
    - SXT family integrative and conjugative elements - **ICE** (SXT ICE, ICEVchHai1)
- MDR examples:
  - O1 AFR13 strain with multiple AMR genes on *IncC* plasmid in Zimbabwe 2018, Yemen 2018-19, Lebanon 2022
  - O1 AFR13 strain in Kenya 2022-23 contained:
    - An ***IncC*** plasmid identical to that from Yemen 2028-19, Lebanon 2022-23, and Mayotte (2023).
    - **ICEVchInd5** (an SXT/R391-like ICE, with a deletion)
    - Resistant to 8/11 AMs tested: cefotaxime, ceftazidime, cefepime, ampicillin, azithromycin, trim/sulfa, nalidixic acid. Also, almost 80% showed reduced susceptibility to imipenem, and 50% to amox/clav.

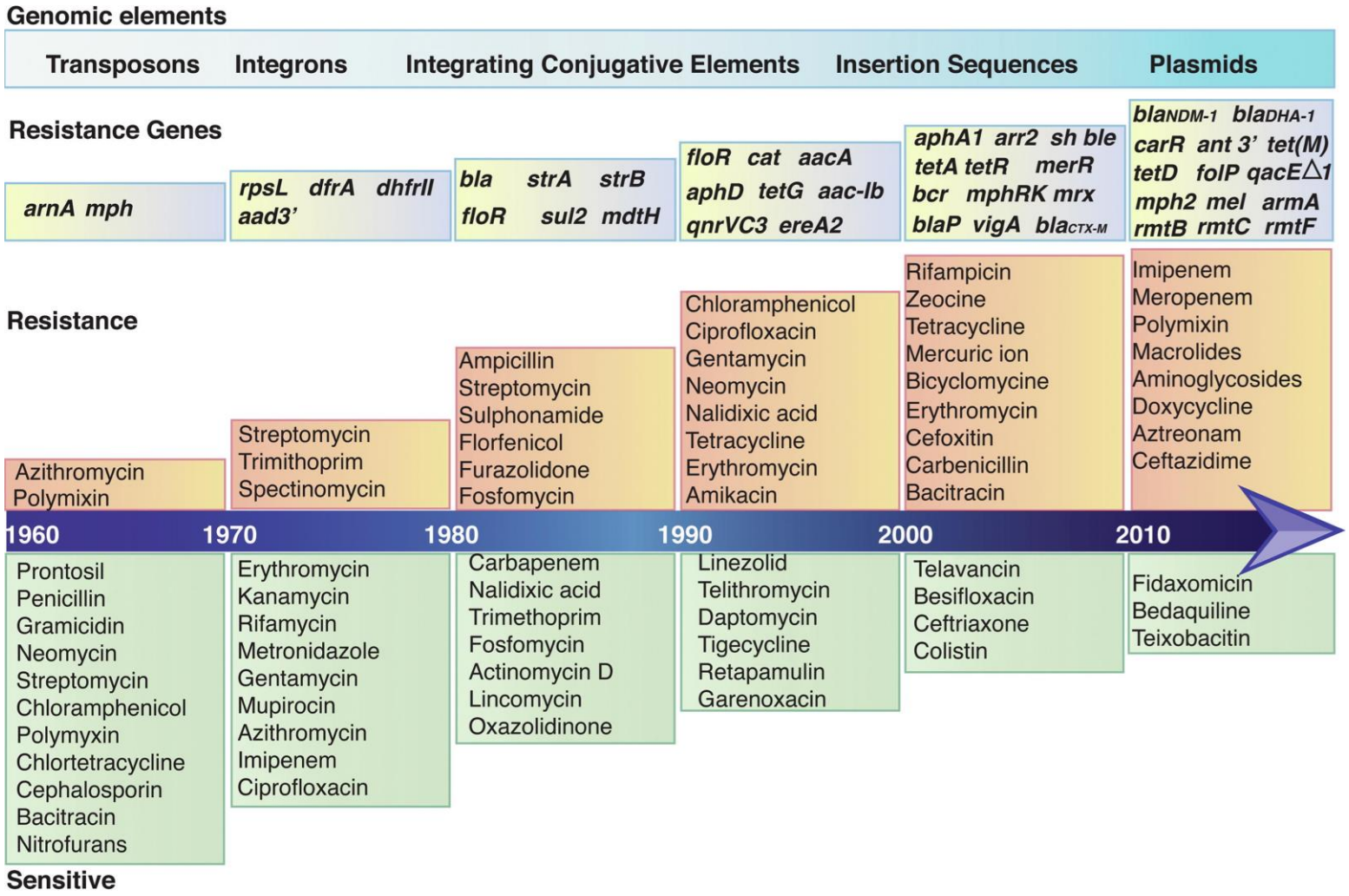
Rise of AMR genes from the 60s

Year	Resistance genes	Sensitive	Resistance
1960–1970	*arnA, mph*	Prontosil, Penicillin, Gramicidin, Neomycin, Streptomycin, Chloramphenicol, Polymyxin, Chlorotetracycline, Cephosporin, Bacitracin and Nitrofurans	Azithromycin and Polymixin
1971–1980	*rpsL, dfrA, dhfrII, aad3*	Erythromycin, Kanamycin, Rifamycin, Metronidazole, Gentamycin, Mupirocin, Azithromycin, Imipenenem and Ciprofloxacin	Streptomycin, Trimethoprim and Spectinomycin
1981–1990	*bla, strA, strB, floR, sul2, mdtH*	Carbapenem, Nalidixic acid, Trimethoprim, Fosfomycin, Actinomycin D, Lincomycin and Oxazolidinone	Ampicillin, Streptomycin, Sulphonamide, Florfenicol, Furazolidone and Fosfomycin
1991–2000	*floR, cat, aacA, aphD, tetG, aac-lb, qnrVC3* and *ereA2*	Linezolid, Telithromycin, Daptomycin, Tigecycline, Retapamulin and Garenoxacin	Amikacin, Erythromycin, Tetracycline, Nalidixic acid, Neomycin, Gentamycin, Ciprofloxacin and Chloramphenicol
2001–2010	*apfA1, arr2, sh-ble, tetA, tetR, merR, bcr, mphRK, mrx, blaP, vigA* and *blaCTX-M*	Telavanacin, Besifloxacin, Ceftriaxone and Colistin	Rifampicin, Bacitracin, Zeocine, Tetracycline, Mercuric ion, Bicyclomycin, Cefoxitin, Carbenicillin and Bacitracin
2011–2015	*blaNDM-1, blaDHA-1, carR, ant 3, tet (M), tetD, folp, qacE1, mph2, mel, armA, rmtB, rmtC, rmtF*	Fidaxomicin, Bedaquiline and Teixobactin	Imipenem, Meropenem, Polymixin, Macrolides, Aminoglycosides, Doxycycline, Aztreonam and Ceftazidime
2016–today	Rv0678, atpE, and pepQ rpoB and rpoC	Fidaxomicin	Bedaquiline[a] and Teixobactin[b, c]

a Derendinger et al. (2023).
b Marchandin et al. (2023).
c Results of in vivo tests.

Das et al. 2020 https://doi.org/10.1016/j.vaccine.2019.06.031
El-Liethy et al. 2025 https://doi.org/10.1016/j.scitotenv.2025.180057

Rise of AMR genes from the 60s – Genomic elements



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Other virulence factors

Toxins

- NAG-specific heat-labile toxin (st)
- HA protease – mucin degradation, facilitates spread
- RTX (rtxA) – cytoskeletal disruption
- hlyA hemolysin – pore-forming toxin
- ace – increases fluid secretion
- zot – loosens tight junctions, promotes invasion

Adherence / Adhesins

- acf – accessory colonisation factors
- ompU / ompT – porins, adhesion and host interaction
- mshA – mannose-sensitive haemagglutinin (type IV pili)
- makA – flagella-mediated cytotoxin

Other mobile elements (Integrons / ICEs)

- Superintegron – large gene reservoir on Chr2

Secretion systems

- Type VI secretion system (T6SS) – bacterial competition, host interaction

Vaccine development

In the 60s injectable with high rate of adverse reactions → Oral vaccines (OCVs).

WHO-prequalified vaccines:

Vaccine	Manufacturer	Status	Composition
Dukoral®	SBL vaccin, Sweden	WHO preq. 2001	Monovalent with killed **O1 strains** and **rCTB**
mORC-Vax™	VaBiotech, Vietnam	Licensed in Vietnam 1997	Bivalent with killed **O1 strains** and one killed **O139 strain**
Shanchol™	Sanofi-Shantha Biotechnics, India	WHO preq. 2001	Same as mORC-Vax™
Euvichol™ / Euvichol-Plus™	Eubiologics, Republic of Korea	WHO preq. 2015 and 2017	Same as Shanchol™
OraVacs™	Shanghai United cell Biotechnology, China	Licensed in China and the Philippines	Similar to Dukoral®
Cholvax™	Incepta, Bangladesh	Licensed in Bangladesh 2020	Same as Shanchol™
CVC 103-HgR (Vaxchora™)	PaxVax Inc., US	Licensed in USA and Europe 2016 and 2020	Attenuated **O1 Classical** strain

Adapted from Montero et al. 2023. https://doi.org/10.3389/fmed.2023.1155751

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