

Evolution of antibiotic resistance in the 7P cholera agent

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Genomic history of the seventh pandemic of cholera in Africa

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11 introductions of 7PET from Asia into Africa

Two main foci (WE and EA)

Elements of periodicity and patterns

Increase in antibiotic resistance of strains



Monitoring antimicrobial resistance

- In 1970, *V. cholerae* O1 biotype ElTor strains presented a natural resistance to polymyxins

- The first antibiotic-resistant isolates were recovered in the early 1980s.

Between 1970 and 1984, 96.4% susceptible isolates
Between 1985 and 1999, 37.6% susceptible isolates
Resistant organisms totaly replaced the susceptible ones after 2000

- The last five introductions events involved multidrugresistant bacteria sublineages



10.0 -

Dynamic of acquisition and accumulation of antibiotic resistance genes

✓ Local acquisitions of ARGs in older lineages

- The acquisition of antibiotic resistance genes (ARGs) were initially caused by multidrug-resistant plasmids of the IncA/C group, encoding resistance to ampicillin, streptomycin, chloramphenicol, sulfonamides, cotrimoxazole, and tetracycline (T1, T5, T6, T10)
- Mutations in the genome: resistance to nalidixic acid is associated to a point mutations in the DNA gyrase gene, gyrA (T5 lineage)

These acquisition events may be linked to non selective mass chemoprophylaxis

- Tanzania in 1977, use of 1.79 tonnes of tetracycline to control an outbreak caused by a susceptible strain; 5 months later, 76% of the isolates had become resistant to it and other antibiotics
- Madagascar in 1999, when doxycycline was used for prophylaxis
- Use of nalidixic acid in the Rwandan refugee camps, which experienced outbreaks of disease caused by Vibrio cholerae O1 and Shigella dysenteriae type 1 in the mid-1990s

Plasmids may be lost in the absence of selective pressure Mutations in the genome are stable and non-transferable

Dynamic of acquisition and accumulation of antibiotic resistance genes

✓ Imports from Asia of multidrug-resistant strains carrying chromosome-encoded antibiotic resistance determinants during the most recent introductions (T8 to T12).

• Acquisition of integrative and conjugative elements

- SXT/R391, a ~100-kb integrative and conjugative element (ICE) encoding resistance to several antibiotics and GI-15)
- Five independent acquisitions of SXT/R391 variants across the global *V.cholerae* phylogeny, each of this five SXT/R391 ICE groups possessing its own set of ARGs.
- Acquired by different groups of wave 2 isolates and the wave 3 isolates
- **Mutations of specific genes**: the *gyrA* gene (S83I) conferring resistance to nalidixic acid (T10) and an additional mutation of the *parC* gene (S85L) conferring decreased susceptibility to ciprofloxacin (T11).

The stability of the SXT chromosomal element was remarkable, with only one isolate of the 531 wave 3 isolates found to lack SXT/R391.

Evolution of antibiotic resistance determinants

Fig. S9

Evolution of antibiotic resistance determinants over time in 7P *V. cholerae* El Tor isolates from Africa and Asia. Panel "A" shows the change in the mean number of antibiotic resistance genes (ARGs) per isolate over time. The number of studied isolates (*n*) is indicated. Panels "B" and "C" show the percentage of isolates containing an IncA/C plasmid or a SXT genomic island, respectively. The analysis was performed for various time periods: 1960-1969, 1970-1979, 1980-1989, 1990-1999, and 2000-2014.



7 PET usual antibiotic resistance

$\mathsf{POL}^\mathsf{R}\,\mathsf{STR}^\mathsf{R}\,\mathsf{SUL}^\mathsf{R}\,\mathsf{TMP}^\mathsf{R}\,\mathsf{SXT}^\mathsf{R}\,\mathsf{TET}^\mathsf{R}\,\mathsf{CHL}^\mathsf{R}\,\mathsf{NAL}^\mathsf{R}\,\mathsf{FT}^\mathsf{R}$

POL^R, marker of EI Tor, modification of lipid A, precise mechanism unknown in WT Vc

- NAL^R, point mutation in the chromosomal gyrA gene
- FT^R, disruption of nitroreductases, precise mechanism unknown in Vc Other resistance determinants carried by the integrative and conjugative element (SXT)



Antibiotic resistance patterns of recent isolates

✓ The Yemeni isolates (2016 and 2017, T13 Lineage) were unexpectedly susceptible to first-line antibiotics:

TMP^R NAL^R FT^R NAL^R: gyrA_S83L

TMP^R: dfrA1 carried by a deleted SXT1

They also were unexpectedly susceptible to polymyxins

Some isolates from Somalia (Lower Juba, 2018) were multiresistant
 STR, SUL, SXT, CHL, AM, TET, EM, FT, NAL, CF, CTX, TMP, ESBL producing
 They were susceptible to polymyxin and certainly are T13 lineage having acquired resistance genes of multiresistant strains circulating concomitantly in bordering countries.

STR, streptomycin, SUL, sulfonamides; SXT, cotrimoxazole; CHL, chloramphenicol; AM, ampilcillin; TET, tetracycline; EM, Erythromycin; FT, nitrofurantoin; NAL, nalidixic acid; CF, cefalotin; CTX, cefotaxim; TMP, trimetoprim



CONCLUSIONS

✓ We are generally observing an accumulation of antibiotic resistance mechanisms over time

- Multiple antibiotic resistance is not necessarily associated with higher virulence an antibiotic-sensitive strain can still cause 1 million cases of cholera
- ✓ Necessity to perform antibioresistance test before initiating the treatment
- ✓ Polymyxins susceptibility should no longer be used as a marker for El Tor