What is the risk of persistent and evolving bacteria in an era of high diphtheria vaccination coverage?

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Full disclosure

- I have no conflicts of interest to declare
- Membership of the National Advisory Committee on Immunization (Canada), the International Expert Committee on the Elimination of Measles, Rubella and Congenital Rubella Syndrome, Pan American Health Organization (PAHO) of the World Health Organization (WHO), and the Measles and Rubella Working Group of the Strategic Advisory Group of Experts (SAGE) WHO
Outline

• Background
  • Methods for literature review
  • Disease, organism and current epidemiology
• Lessons from the Epidemic in the Former Soviet Union (FSU)
• Non-toxigenic and zoonotic strains - implications
• Conclusions
Methods for literature review

- Review of literature - Medline and Embase
- MESH terms. “Diphtheria Toxin” or “Diphtheria” or “Corynebacterium diphtheriae” combined with the following:
  - “Drug Resistance, Multiple, Bacterial” or “Microbial Sensitivity Tests” or “Drug Resistance, Bacterial” “Evolution” “Carrier State” “Disease Outbreaks” or “Disease Reservoirs” or “Disease Transmission, Infectious” “Genetic Variation” or “Genomic Instability” or “Genotype” “Electrophoresis” or “Electrophoresis, gel, pulsed-field” “Zoonoses”
Methods for literature review

**Article inclusion criteria:**
Primary focus on diphtheria, biological evolution, persistent or emerging infection, or changes in disease.

- Medline and Embase (509 articles retrieved)
- 36 articles selected for full text review
- 1 article identified by hand-searching
- 21 articles included in analysis
  - 473 articles excluded following title and abstract review
  - 16 articles excluded criteria following full text review
Diphtheria

- **Organism:** *Corynebacterium diphtheriae* and *C.ulcerans*, virulent strains produce toxin (toxigenic)
  - Gram positive, fermentative, pleomorphic rod
  - *C diphtheriae* has four biovars, *gravis, mitis, intermedius, belfanti* (all may produce lethal exotoxin, *belfanti* less often)
- **Distribution:** worldwide, mainly children, very rare in countries with effective childhood immunisation programme
- **Source:** Nose or throat of human case or carrier through close contact with infected secretions. In tropics through infected skin lesions. *C.ulcerans*: raw milk, cattle, cats, dogs
- **Potential for eradication?** No
Clinical picture

Copyright American Academy of Pediatrics [http://www.vaccineinformation.org/photos/diphaap002.jpg]
Courtesy of Centers for Disease Control and Prevention [http://www.vaccineinformation.org/photos/diphiac001.jpg]
Cutaneous diphtheria signs/symptoms

- Chronic non-healing rolled-edge ulcers with base covered by hard, grey adherent membrane
- Usually in travellers returning from tropics
- Often follow insect bites
- Staph and strep commonly isolated as well
- Toxic manifestations rare
- As infectious as respiratory diphtheria - possibly more so
Diphtheria toxin

• Diphtheria genome is very stable, indicating changes through gene gain/loss and nucleotide substitution (versus intragenic reshuffling)

• No microbial factors distinguish epidemic from non-epidemic strains apart from toxin production (Mokrousov 2009)

• Other pathogenic and virulence factors exist
  • Non-toxigenic strains can cause invasive disease, but not epidemics
  • *C. ulcerans* can be toxigenic but does not cause epidemics

• Diphtheria toxin A subunit is highly conserved; B subunit has more variability
  • Selection advantage may be through increasing transmission of the organism
Current epidemiology of diphtheria

• 2012 global figures
  • 4,489 reported cases and 2500 estimated deaths (in 2011)
  • 83% estimated DTP3 coverage - 32% of countries reached >=80% DTP3 coverage in all districts

• High coverage countries
  • Virtually no cases, persistent non-toxigenic strains, possible increase in diversity of stains
  • Re-introduction can lead to clonal outbreaks, and possible transfer of toxin to local strains

• Low coverage countries
  • Continuous circulation in some regions
<table>
<thead>
<tr>
<th>Country</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>India</td>
<td>2525</td>
</tr>
<tr>
<td>Indonesia</td>
<td>1192</td>
</tr>
<tr>
<td>Iran</td>
<td>150</td>
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<tr>
<td>Nepal</td>
<td>138</td>
</tr>
<tr>
<td>Lao People's Democratic Republic</td>
<td>130</td>
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<tr>
<td>Pakistan</td>
<td>98</td>
</tr>
<tr>
<td>Somalia</td>
<td>65</td>
</tr>
<tr>
<td>Thailand</td>
<td>63</td>
</tr>
<tr>
<td>Myanmar</td>
<td>19</td>
</tr>
<tr>
<td>Sudan</td>
<td>18</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>16</td>
</tr>
<tr>
<td>Angola</td>
<td>15</td>
</tr>
<tr>
<td>Viet Nam</td>
<td>12</td>
</tr>
<tr>
<td>Germany</td>
<td>9</td>
</tr>
<tr>
<td>Eritrea</td>
<td>8</td>
</tr>
<tr>
<td>Latvia</td>
<td>8</td>
</tr>
<tr>
<td>Russian Federation</td>
<td>5</td>
</tr>
<tr>
<td>Ukraine</td>
<td>5</td>
</tr>
<tr>
<td>Iraq</td>
<td>3</td>
</tr>
<tr>
<td>Namibia</td>
<td>2</td>
</tr>
<tr>
<td>Sweden</td>
<td>2</td>
</tr>
<tr>
<td>Belgium</td>
<td>1</td>
</tr>
<tr>
<td>Canada</td>
<td>1</td>
</tr>
<tr>
<td>Congo</td>
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<td>Madagascar</td>
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<td>Netherlands</td>
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<td>Turkmenistan</td>
<td>1</td>
</tr>
<tr>
<td>USA</td>
<td>1</td>
</tr>
</tbody>
</table>
Immunization coverage with DTP3 vaccines in infants (from <50%), 2012

Date of slide: 16 July 2013

http://www.who.int/immunization_monitoring/diseases/diphtheria/en/
Diphtheria, cases and deaths
England and Wales, 1914-2012

Notifications
Deaths

Routine vaccination

* notifications up to 1985, laboratory confirmed cases 1986-2012

Courtesy of Public Health England
https://www.gov.uk/government/organisations/public-health-england
Immunity to diphtheria in adults is quite low despite good control

**Percent of sera with antitoxin => 0.1 iu/ml**

- 15-34 yrs
- 35-54 yrs
- 55 yrs+

2862 samples collected from 6 PHLs in England, 1991

*Maple PA et al Vaccine 2000*
Typing methods

• Multilocus Sequence Typing (MLST) becoming the standard (used in 2/8 EU centres in 2010)

• Ribotyping offers good discrimination reproducibility (used in 6/8 EU centres in 2010)

• Other methods: Pulsed-field gel electrophoresis (PFGE), Random amplification of polymorphic DNA (RAPD), Amplified fragment length polymorphism (AFLP), Multilocus enzyme electrophoresis (MEE), Spoligotyping, Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry

• Application relies on good public health laboratory-surveillance, partnerships and expertise in lab diagnosis (WHO, ELWGD, Dipnet, EDSN (European Diphtheria Surveillance Network)

Diphtheria in Former Soviet Union (FSU) 1990-1997
Origins of FSU epidemic was not vaccine-driven evolution

- Increased adult susceptibility
- Increased susceptibility of children
- Inadequate information of physicians and the public (false contraindications for vaccine)
- Dissolution of the Soviet Union leading to socioeconomic problems, delays in control measures, damaged health infrastructure, large population movements
- Role of the military – not routinely immunized; demobilization of 100,000 troops in 1988-89
- **Vaccine effectiveness remained high**
Patterns of FSU outbreak

- Pre-epidemic period – simultaneous presence of many different electrophoretic types (ETs)
- In 1970s to 80s (peak 1983) biotype changed from gravis to mitis
- By 1990s *gravis* was predominant, but *mitis* also found
- Epidemic clonal group had a unique PFGE profile and comprised MEE-defined ET8 complex strains of D1 (Sankt Petersburg) or D4 ribotypes (Rossija) regardless of their biotype
- Consistent with introduction of new strain, emergence of strain and/or acquisition of toxin by non-toxigenic strains
Distribution of
(A) Ribotypes and
(B) Electrophoretic
types in Russia
1985-97
Popovic et al JID 2000
Ribotypes D4 and D7 were documented in Russia before the epidemic; D7 was predominant.

During the epidemic D1 and D4 accounted for >80% isolates.
Impact of restoring vaccine programs: Evolution of organism post-epidemic in FSU: Belarus

• In Belarus changes occurred in the predominant types between 1996-2005 as the epidemic waned

• *C. diphtheriae* biovar *gravis* was replaced by *mitis*, and toxigenic strains fell from 47.1% to 5.8% of isolates. D1 (Sankt Petersburg) fell from 24.3 to 2.3%; D4 (Rossija) increased from 25.1 to 49.1%

• Non-toxigenic isolates with D10 (Cluj) and D4 (Rossija) ribotypes increased to 49.3 and 30.1%

• *Kolodka* et al *BMC Infect Dis* 2006
Lessons learnt from European post-epidemic carriage study

<table>
<thead>
<tr>
<th>C. diphtheriae</th>
<th>Estonia N=4505</th>
<th>Latvia N=2480</th>
<th>Lithuania N=2988</th>
<th>Turkey N=2771</th>
<th>UK N=8551</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxigenic (per 10,000)</td>
<td>0</td>
<td>8</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Non-toxigenic (per 10,000)</td>
<td>2</td>
<td>28</td>
<td>13</td>
<td>40</td>
<td>4</td>
</tr>
</tbody>
</table>

- Carriage of toxigenic strains is very rare in countries where diphtheria has been controlled (zero in Bulgaria, Finland, Greece, Italy, Ireland)
- Isolation of non-toxigenic strains may be more likely in countries that are also identifying toxigenic strains
  - 2 isolates in Lithuania were non-toxigenic toxin gene-bearing (NTTB)

Wagner et al Clin Microbiol Infect 2010
Toxigenic *C. diptheriae* persist in at-risk populations/environments

- Dendrogram of 68 strains collected from 8 countries in 5 regions shows 3 clusters:
  - Russian/NIS ET8 cluster
  - Australian cluster from 1992
  - US and Canadian (Ontario) isolates from patients and carriers, 1973-96 clearly distinct from Russian/NIS isolates and more diverse both phenotypically and genotypically.
  - Clustering of older and more recent isolates suggests that an endemic focus may have persisted for >25 years

- *Popovic JID 2000, Marston et al J Clin Micro 2001*
Non-toxigenic strains in countries with high vaccine coverage

- Risk factors include homelessness, alcoholism, injecting drug use, diabetes mellitus, skin infections, poor dental hygiene.

- UK: Ribotyping found a single strain never associated with toxigenicity (*Reacher et al 2000*).
  - Upward trend probably due to increased ascertainment. Exclusively found in throat, >25% associated with beta haemolytic streptococci
  - No evidence of reversion/acquisition of toxigenicity

- Italy: Non-toxigenic strains isolated in Italy, distinct ribotypes from Russian/NIS strains (*Von Hunolstein J Med Micro 2003*)

- Canada: Invasive clone identified in urban poor in Vancouver (*Romney et a J Clin Micro 2006*).

- Poland: Increase in non-toxigenic single clone (*Zasada et al IJID 2010*)
42 Non-toxigenic invasive strains in France, New Caledonia, Poland typed by MLST Farfour et al J Clin Micro 2011

- Diverse: 11 STs
- Geographically predominant, for varying time periods
- Strains in Poland ST8 same as FSU epidemic
Zoonotic diphtheria - a potential reservoir?

- Increasing identification of *C. ulcerans* – case reports, no epidemic potential, mostly zoonotic transmission, only possible person to person transmission reported.

Toxigenic strains in UK 1986-2008

*Wagner K et al Epidemiol Infect 2010*
Conclusions

- What is the risk of persistent and evolving bacteria in an era of high diphtheria vaccination coverage? **100%**
- Could vaccine-derived selective pressure be contributing to emergence?
  - Vaccine effectiveness remains high
  - Epidemics have not occurred where vaccine coverage is high
  - Increased non-toxigenic strains and *C. ulcerans* - greater ascertainment?
- Toxin is necessary but not sufficient (*C. ulcerans*) for epidemic potential
  - May underline the importance of understanding determinants of **transmission** as well as virulence
THANK YOU FOR LISTENING