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Typhoid fever: update and new insides

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Introduction

I will focus on Typhoid fever caused by solely *Salmonella* ser. Typhi

I will not explicitely discuss:

- ((Para) Typhoid) fever caused by *Salmonella* ser. Paratyphi A, B or C
- Invasive non typhoidal Salmonellosis

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Introduction

Typhoid fever is a global health problem

in 2017: 11 million cases

116 000 deaths

Physical illness and death

- (adverse pregnancy outcome)
- impair physical and cognitieve development
- school attendance and performance
- limit workproductivity

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Introduction

Typhoid fever is a bacterial blood infection

Affecting mostly children

- Southeast Asia
- Sub-Saharan Africa

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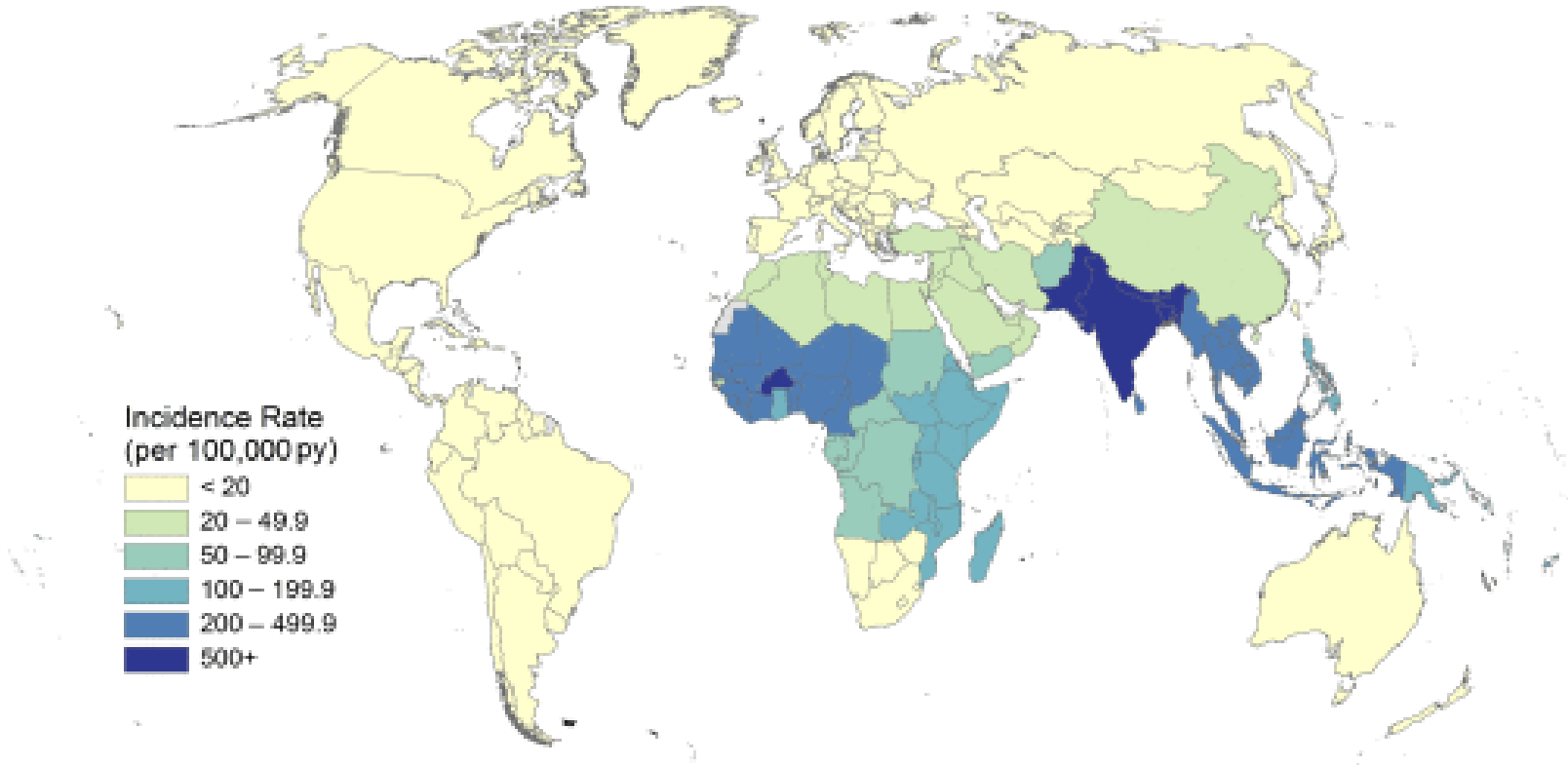
Epidemiology



Estimated incidence of typhoid and paratyphoid fevers by country per 100,000 population, 2015

Radhakrishnan A et al. ,Am. J. Trop. Med. Hyg., 99(Suppl 3), 2018, pp. 4–9

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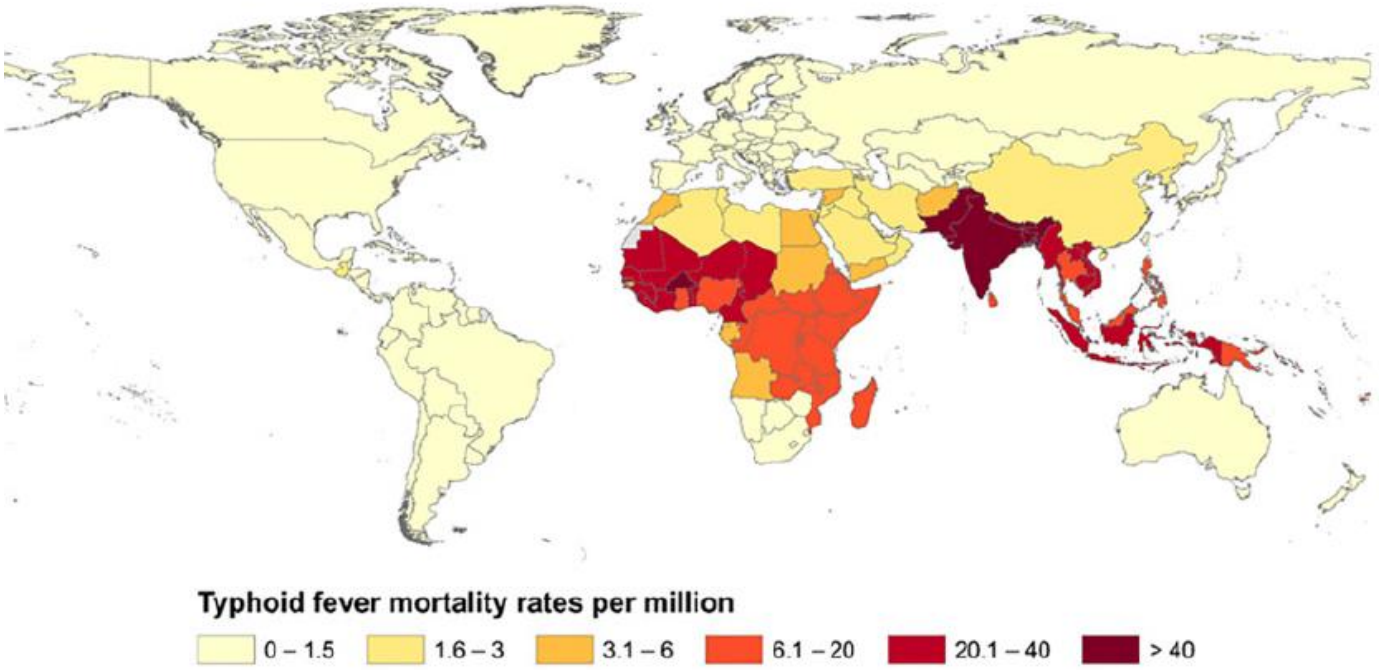
Epidemiology



Radhakrishnan A et al. ,Am. J. Trop. Med. Hyg., 99(Suppl 3), 2018, pp. 4–9

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Epidemiology

- Africa was previously estimated to have a moderate (10-100 cases/100 000) incidence
- High incidence in children <15 years (2-14y)
- Rural populations having similar of higher incidence than urban locations

Baker S. et al. CID 2016;62(S1):S1-3

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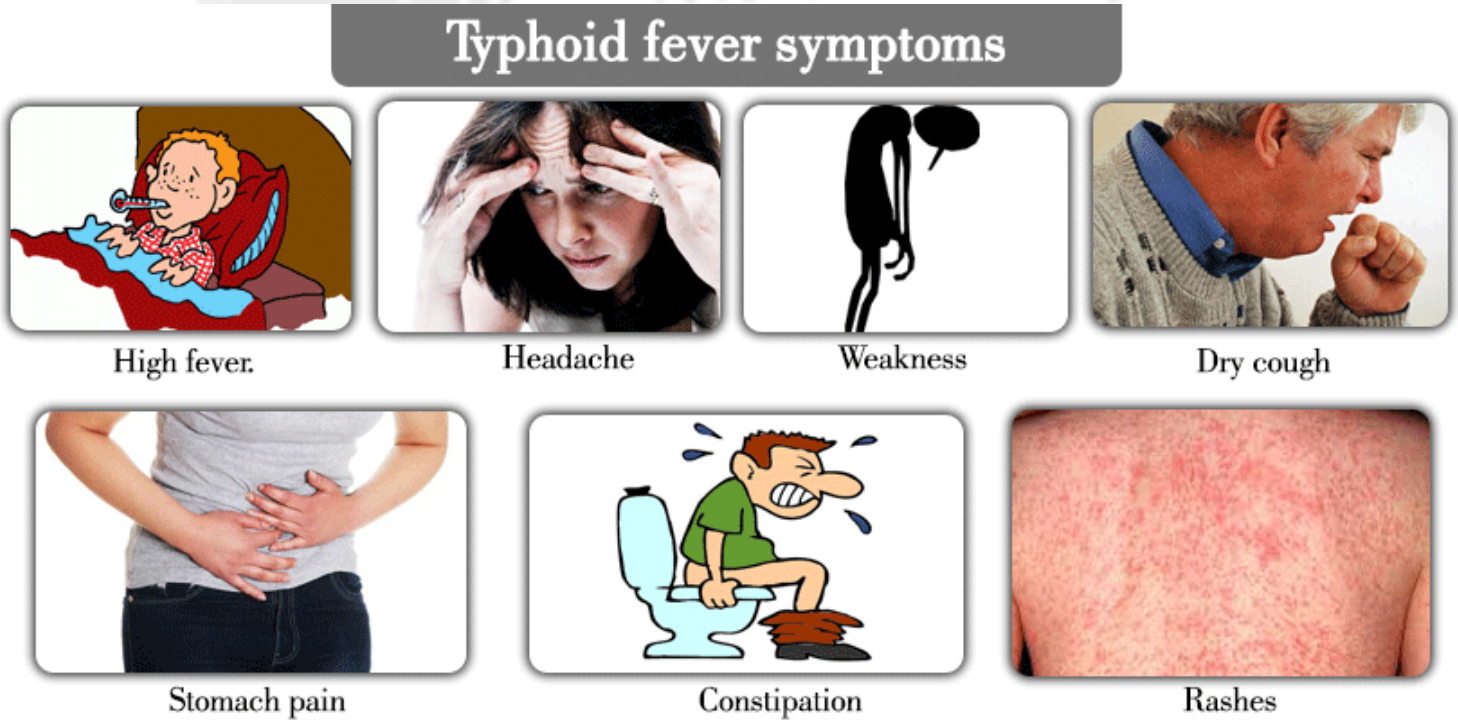
Clinical presentation

- Non specific symptoms

  Not distinguishable from other febrile ilnesses



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Clinical presentation

- Incubation period: 7 – 14 days

- stepwise fever pattern: characterized by a rising temperature over the course of each day that drops by the subsequent morning. The peaks and troughs rise progressively over time.

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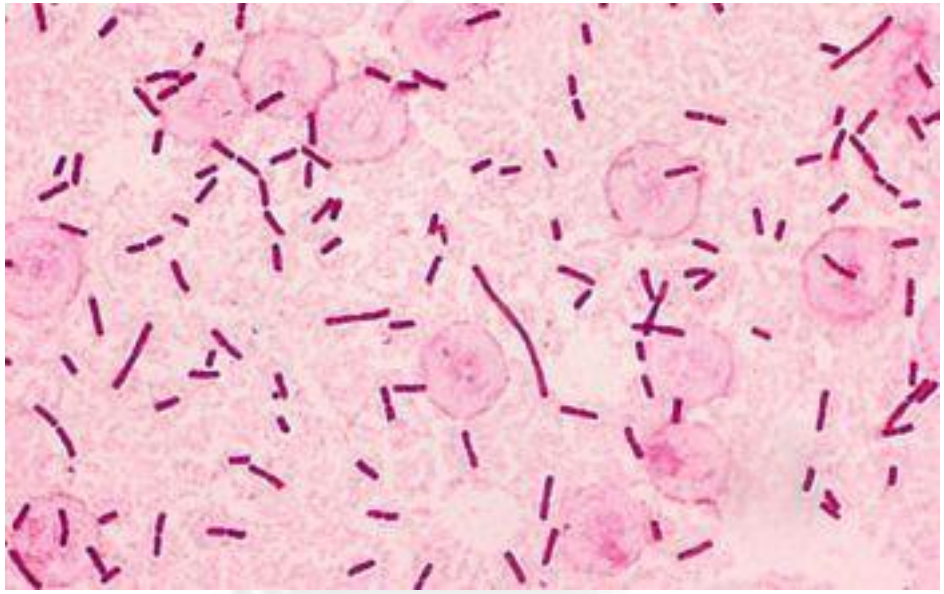
Bacteriology

- *Salmonella enterica* subspecies *enterica* serovar Typhi
  - *Salmonella* Typhi
- *Enterobacteriaceae*
- *Gram negative, rod*



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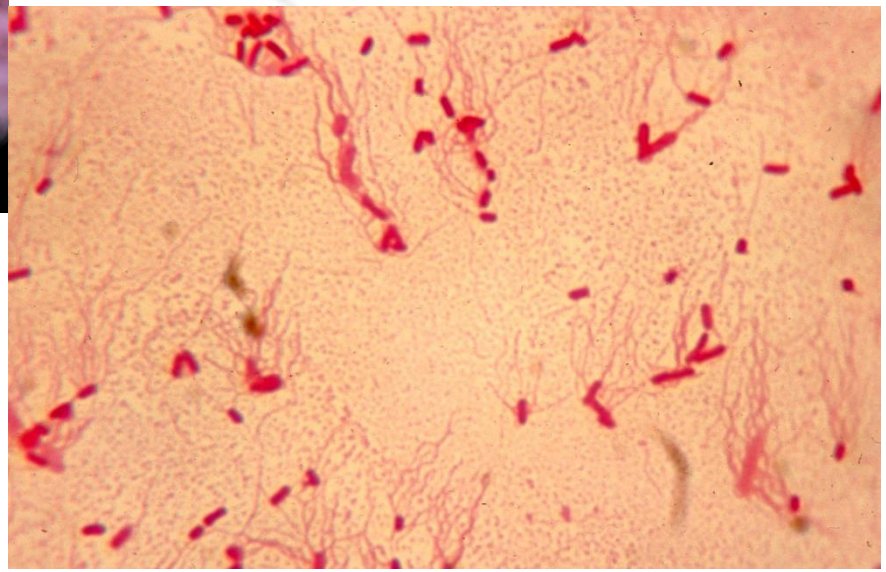
Bacteriology



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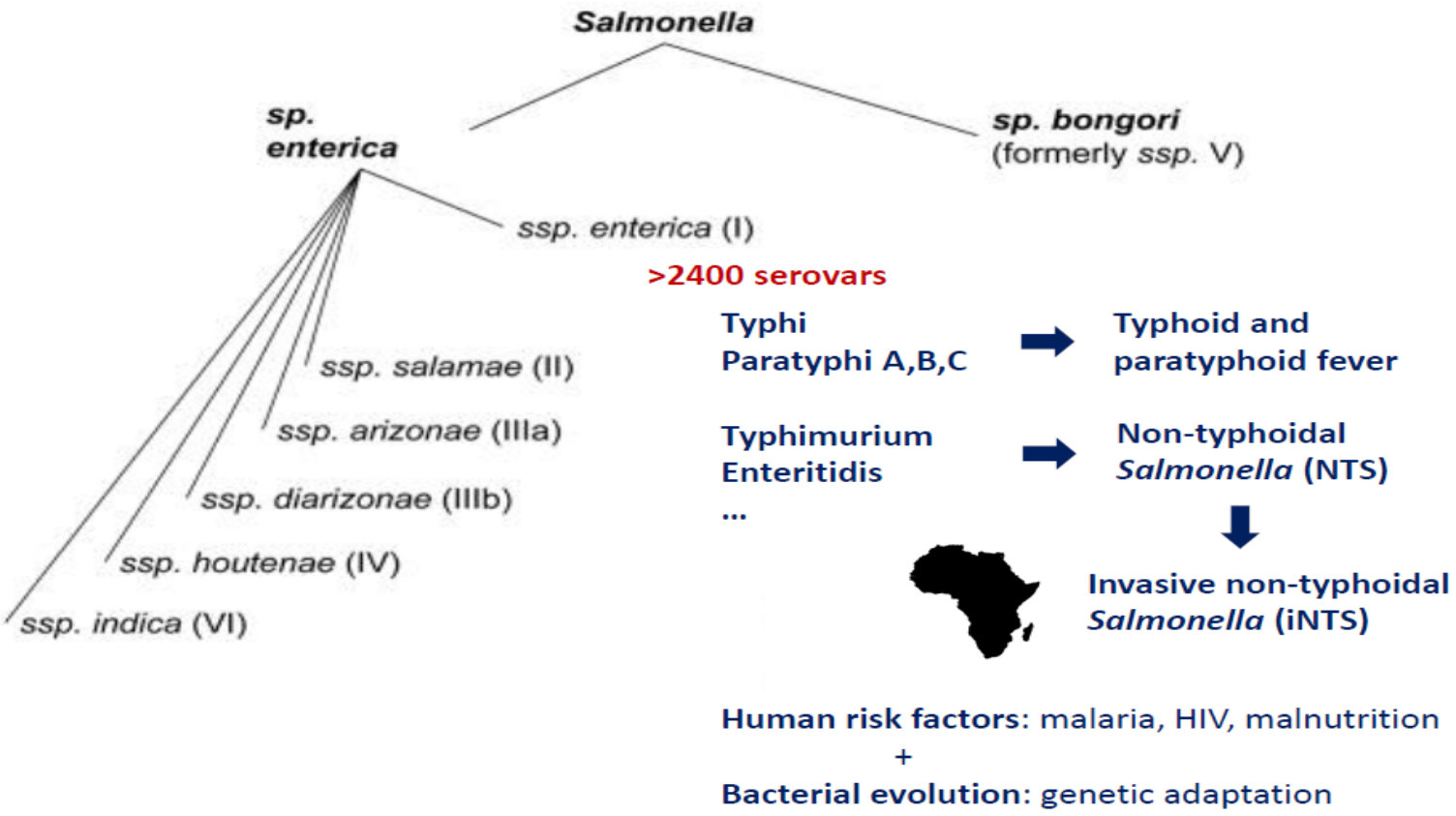
Bacteriology

Salmonella: >2400 different serovars



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Pathophysiology

- *S. typhi* enters the digestive system
- In gut it crosses the intestinal barrier
- Disseminated through the body
- *S. typhi* is taken up by macrophage cells, where it remains avoiding the activation of the inflammatory response
  - Evades immune system by producing Vi polysaccharide coat

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Bacteriology

- *S. typhi* produces a toxin protein when inside human cells
- Latency and multiplication in lymphnodes, spleen, liver, bone marrow
- Via gallbladder in stool

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Transmission

Faeco-oral

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Clinical diagnosis

non specific symptoms:

often mistaken for malaria, influenza, dengue fever, pneumonia, tuberculosis, brucellosis,….

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Laboratory diagnosis
Culture

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Laboratory diagnosis
Culture

- Reference assay: bone marrow culture, highest sensitivity
- Blood culture:
  - Blood collected prior AB administration
  - As early as possible in the course of disease
  - Bacterial load is low in acute typhoid <1CFU/mL of blood, maximal during first week of illness
  - Optimal volume of blood, more is better
  - Sensitivity of blood culture 40-60%

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Laboratory diagnosis
Serology

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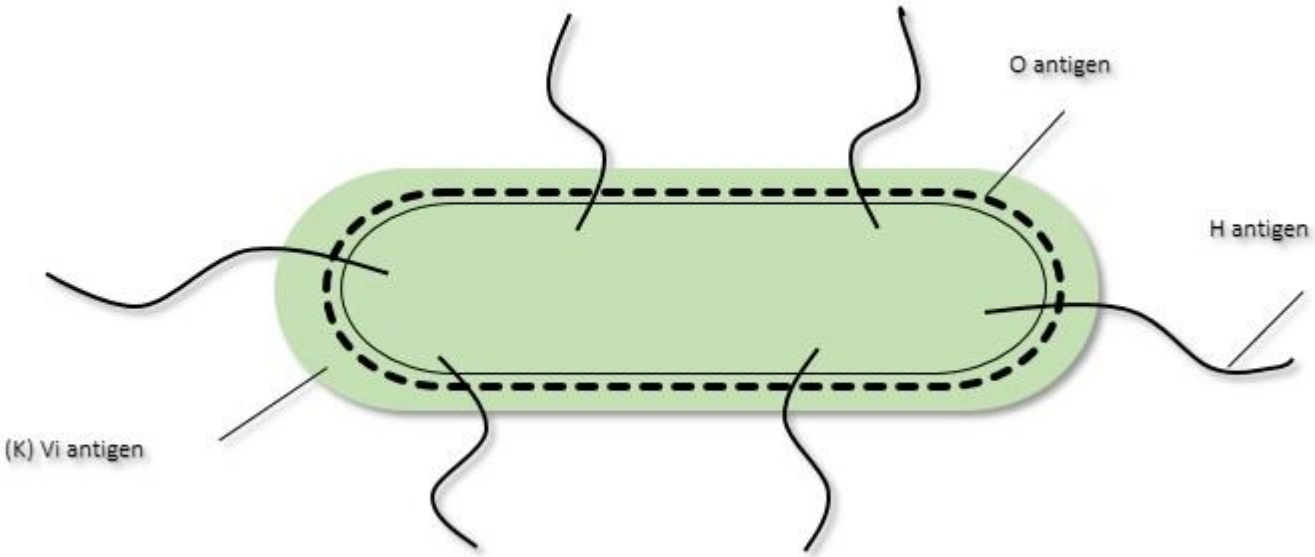
Laboratory diagnosis
Serology



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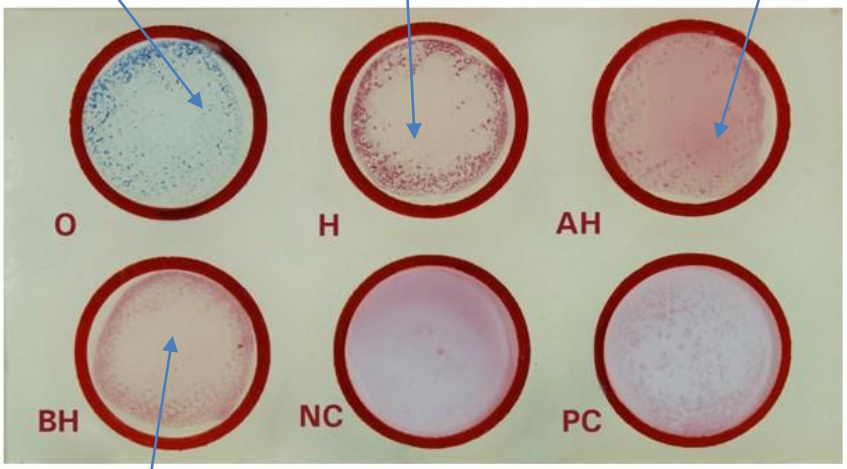
Laboratory diagnosis
Serology

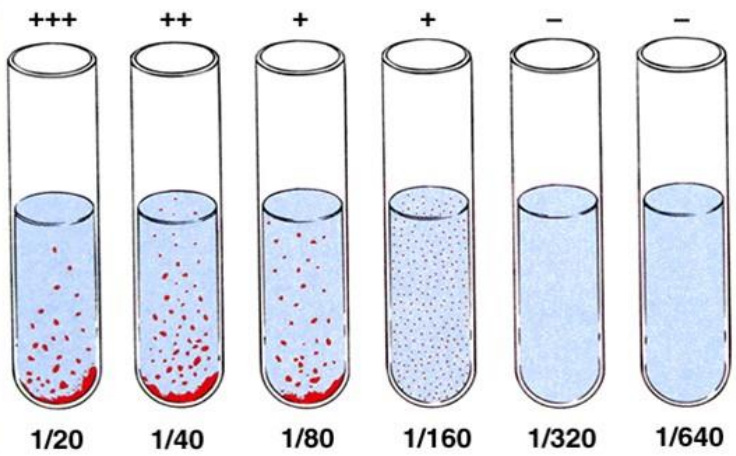
cell wall

flagella

flagella paratyphi A

Widal Test





Rapid Slide Test

Quantitative Tube Test

flagella paratyphi B

www.onlinebiologynotes.com

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Laboratory diagnosis
Serology

Interpretation, example

Normal: O<1:80, H<1:160

Typhoid fever: O ≥1:80, H ≥1:160

Early infection: O ≥1:80, H <1:160

Vaccination or nonspecific memory reaction: O <1:80, H ≥1:160

Laboratory diagnosis serology

Hoffman et al. stated that the results of single Widal test, tube dilution or slide agglutination test are virtually un-interpretable unless the sensitivity and specificity of the test for the specific laboratory and patient population are known [12]. Olopenia and King stated that the value of Widal test depends upon the standardization and maintenance of the antigens to produce consistent results. They also mentioned that even since 1936 when

Bakr *et al. Annals of Clinical Microbiology and Antimicrobials* 2011, **10**:7

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Laboratory diagnosis serology

A) **Remel:** Remel stained *Salmonella* O and H suspensions, Remel Europe ltd, UK
B) **BioSystems:** Febrile serodiagnostic agglutination slides and tubes, Biosystems S A, Barcelona, Spain
C) **Dialab:** Bacterial agglutination test, Dialab, Austria
D) **Biotec:** Stained bacterial antigen suspensions, Biotec, UK

comparator

ELISA test (*Salmonella* Typhi IgM anti-LPS ELISA BIO-QUANT INC, USA)

Bakr *et al. Annals of Clinical Microbiology and Antimicrobials* 2011, **10**:7

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Laboratory diagnosis serology

Table 5 Sensitivity, specificity and accuracy of the 4 Widal brand for anti-O antibodies of 91 random serum samples of the 150 clinically diagnosed typhoid fever cases using IgM anti-LPS ELISA as a reference test at three cut-off values

Using a cut-off 1/80			
Brand	**sensitivity**	**specificity**	**accuracy**
Remel	100.00%	54.16%	87.91%
Biosystems	100.00%	58.33%	89.01%
Biotech	97.00%	58.33%	86.81%
Dialab	88.10%	91.66%	89.01%
Using a cut-off 1/160			
Remel	89.50%	83.33%	87.91%
Biosystems	83.58%	95.83%	86.81%
Biotech	80.59%	83.33%	81.31%
Dialab	47.76%	95.83%	60.40%
Using a cut-off1/320			
Remel	59.70%	100.00%	70.32%
Biosystems	46.26%	100.00%	60.43%
Biotech	35.82%	100.00%	52.74%
Dialab	2.89%	100.00%	28.57%

Table 9 Sensitivity, specificity and accuracy of the 4 Widal brand for anti-H antibodies of 91 random serum samples of the 150 clinically diagnosed typhoid fever cases using IgM anti-LPS ELISA as a reference test at three cut-off values

Widal Brand	sensitivity	specificity	accuracy
Using a cut-off 1/80			
Remel	94.02%	8.33%	71.42%
Biosystems	91.04%	16.66%	71.42%
Biotech	86.56%	20.83%	69.23%
Dialab	73.13%	45.83%	65.93%
Using a cut-off 1/160			
Remel	83.58%	20.83%	67.03%
Biosystems	80.59%	29.16%	67.03%
Biotech	73.13%	54.16%	68.13%
Dialab	55.22%	91.66%	64.83%
Using a cut-off 1/320			
Remel	56.71%	79.16%	62.63%
Biosystems	47.76%	74.07%	57.14%
Biotech	35.82%	91.66%	50.54%
Dialab	19.40%	100.00%	40.65%

Bakr *et al. Annals of Clinical Microbiology and Antimicrobials* 2011, **10**:7

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Laboratory diagnosis serology

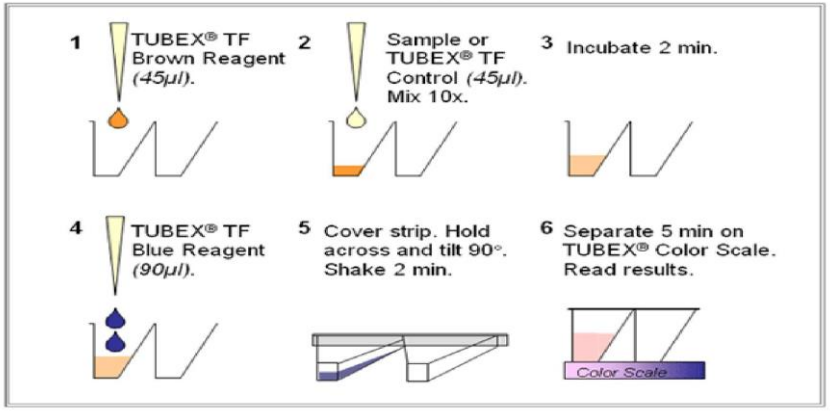
Recommendation

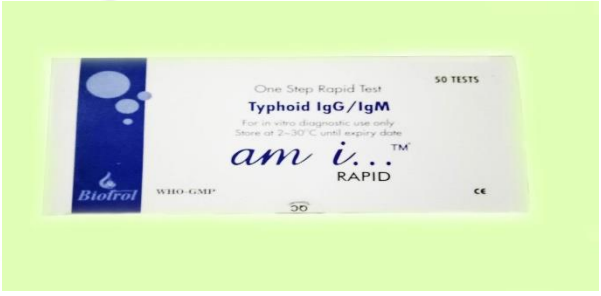
- An effort must be done to establish a protocol for the standardization of the different commercially available Widal brands to ensure consistent results by different brands.
- Till the achievement of this standardization, we recommend the dual use of two Widal brands to improve the sensitivity and specificity of the test, first by screening sera with a highly sensitive brand and second by tracing false positive cases by testing positive sera in the first testing by a highly specific brand.
- If only one brand is to be used, the cut-off value of this brand must be determined to the community population.
- We recommend the use of acute phase sera using Widal test to diagnose typhoid fever cases and not rely on a 4 fold increase in the antibodies titer using paired sera.

Bakr *et al. Annals of Clinical Microbiology and Antimicrobials* 2011, **10**:7

Laboratory diagnosis serology

TUBEX® TF Assay procedure





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Laboratory diagnosis
Serology

Evaluation of Serological Diagnostic Tests for Typhoid Fever in Papua New Guinea Using a Composite Reference Standard

Valentine Siba,[a] Paul F. Horwood,[a] Kilagi Vanuga,[b] Johanna Wapling,[a] Rebecca Sehuko,[a] Peter M. Siba,[a] and Andrew R. Greenhill[a]*
Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea,[a] and Goroka General Hospital, Goroka, Papua New Guinea[b]

Evaluation of:

- TubexTF, IDL Biotech, Sweden
- TyphiDot, Reszon Diagnostics, Malaysia
- TR-02, Prototype, Reszon Diagnostics, Malaysia, immunochromatographic IgM
- Widal, Remel

Reference assay:

- Blood culture
- PCR on whole blood DNA

Positive: culture OR PCR positive Negative: culture AND PCR negative

November 2012 Volume 19 Number 11 Clinical and Vaccine Immunology p. 1833–1837

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Laboratory diagnosis
Serology

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Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea,[a] and Goroka General Hospital, Goroka, Papua New Guinea[b]

Results: N=500
20 culture positive
40 PCR positive (15 culture positive)

TABLE 1 Sensitivity, specificity, PPV, and NPV of typhoid fever diagnostic tests, using blood culture and a composite reference standard (blood culture and real-time PCR) as comparators

Test	% (95% confidence interval)			
	Sensitivity	Specificity	PPV	NPV
Blood culture				
Tubex	77.3 (59.8–94.8)	87.4 (84.5–90.4)	0.221 (0.128–0.313)	0.988 (0.978–0.998)
TyphiDot	95.5 (86.8–104)	79.1 (75.4–82.7)	0.174 (0.106–0.241)	0.997 (0.992–1.00)
TR-02	100	81.6 (85.1–78.1)	0.200 (0.125–0.275)	1
Widal (titer, 160)	86.4 (72.0–100.7)	95.0 (93.0–96.9)	0.442 (0.293–0.590)	0.993 (0.986–1.001)
Blood culture plus PCR (composite reference standard)				
Tubex	51.1 (36.8–65.4)	88.3 (85.3–91.2)	0.312 (0.208–0.415)	0.946 (0.924–0.967)
TyphiDot	70.0 (52.4–79.5)	80.1 (76.5–83.8)	0.256 (0.178–0.334)	0.958 (0.938–0.978)
TR-02	89.4 (80.5–98.2)	85.0 (81.7–88.3)	0.382 (0.291–0.473)	0.987 (0.976–0.998)
Widal (titer, 160)	51.1 (36.8–65.4)	95.8 (94.0–97.7)	0.558 (0.410–0.707)	0.950 (0.930–0.970)

November 2012 Volume 19 Number 11 Clinical and Vaccine Immunology p. 1833–1837

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Laboratory diagnosis Serology

Rapid diagnostic tests for typhoid and paratyphoid (enteric) fever

Lalith Wijedoru[1], Sue Mallett[2], Christopher M Parry[1]

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Test name	Sensitivity (95% CI)	Specificity (95%CI)
TUBEX	78% (71% to 85%)	87% (82% to 91%)
Typhidot (any form)	84% (73% to 91%)	79% (70% to 87%)
Test-it (any form)	69% (59% to 78%)	90% (78% to 93%)

The RDTs evaluated are not sufficiently accurate to replace blood culture as a diagnostic test

A Systematic Review and Meta-Analysis of the Performance of Two Point of Care Typhoid Fever Tests, Tubex TF and Typhidot, in Endemic Countries

Kamala Thriemer*, Benedikt Ley, Joris Menten, Jan Jacobs, Jef van den Ende
Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

PLoS ONE 8(12): e81263. doi:10.1371/journal.pone.0081263

Conclusion

The performance of Typhidot and TUBEX TF does not support the use of either rapid diagnostic test exclusively as a basis for diagnosis and treatment. Although more time consuming and related to higher expenses and logistics, blood culture and molecular biologic techniques remain the reference method of choice, despite its limitations. There is a need to develop an RDT for typhoid fever that has a performance level comparable to malaria RDTs.

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Treatment

Antibiotic resistance in *Salmonella*: definitions

MDR **Multidrug resistance**

Co-resistance to the first-line antibiotics:
- **ampicillin**
- **chloramphenicol**
- **trimethoprim-sulfamethoxazole**

ESBL **Extended-spectrum beta-lactamases**

- **Hydrolysis of β-lactam antibiotics by β-lactamase enzymes (Gram-neg bacteria)**
- **β-lactamases: penicillins**
- **Extended-spectrum β-lactamases: penicillins, cephalosporins, carbapenems, …**
- **Functional classes A, B, C, D**

DCS **Decreased Ciprofloxacin Susceptibility**

- **DCS = Low level fluoroquinolone resistance (0.064µg/mL < MIC < 1 µg/mL)**
  → Treatment failure in *Salmonella*
  → 4th generation gatifloxacin remains efficacious
- **Full resistance = ciprofloxacin MIC ≥ 4µg/mL (CLSI2011)**
  → Rare in *Salmonella* (only reported in Asia)

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Treatment

- MDR: first appeared in 1970

- MDR genotype H58, originated in 1990s and spread from South Asia to Southeast Asia and Africa

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Treatment

- Recommended

  If no complications: Ciprofloxacin P.O (however cipro R is increasing)

  If complications (peritonitis, intestinal bleeding) : Ceftriaxone I.M

  Reserve: Azithromycin

  Does not work: gentamycin, cefalosporin I and II even if S on disk testing

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Treatment

- If untreated can lead to complications such as intestinal perforation, or death (10-20%)

- 1-4% of patients recover but continue as asymptomatic carriers

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Typhoid Mary

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Typhoid Mary

Brief period of asymptomatic faecal shedding

A subset of patients will progress to long term asymptomatic carriers

Risk factor for carriage : gallstones

Residual bacterial infection is often located in gallbladder (need for surgery, higher AB dosage)

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Prevention
WASH

Control measures for the management of typhoidal *Salmonella*

Level	Interventions
Water and sanitation infrastructure	Ready access to potable water
	Use of improved sanitation
	Sewage collection and treatment
Health systems	Accurate, rapid diagnosis, and antimicrobial susceptibility testing
	Identification and treatment of chronic carriers
	Appropriate antimicrobial treatment
	Vaccination*
	Food safety regulations, implementation, and enforcement
Education	Handwashing before eating and before food preparation and after defecation[20]
	Food safety education

*Vaccines for paratyphoid fever are not available.

Radhakrishnan A et al. ,Am. J. Trop. Med. Hyg., 99(Suppl 3), 2018, pp. 4–9

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Prevention
Vaccines

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Prevention

WHO recommends following 3 vaccines:

- Ty21a vaccine: oral live attenuated in capsule formulation
  - > 6 years of age

- Vi-PS vaccine: injectable unconjugated vaccine
  - Unconjugated polysaccharide based on purified Vi antigen
  - From 2 years

- TCV: injectable typhoid conjugate vaccine
  - Vi polysaccharide antigen linked to tetanus toxoid protein
  - From 6 months – 45 years
  - Expected longer duration of protection

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Conclusion

Africa is facing several threats :

- Difficulties to diagnose
  - Difficult to estimate the burden of TF cases
  - Treatment without diagnosis, increased risk of R development
- Travel and import of R strains
- Growing and denser cities, increased risk
- Climate change

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- Antibiotic resistance, travel
- Denser cities
- Climate change
- Difficult to diagnose

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Conclusion

But there is HOPE:

- Call for POCT development
- Prevention is possible through
  - WASH
  - Better vaccines with increased immunoprotection and from 6 months of age
- Other treatment option are explored e.g. bacteriophages

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Thanks to

I thank my ex-colleagues from

ITM, Belgium for sharing some of their experiences

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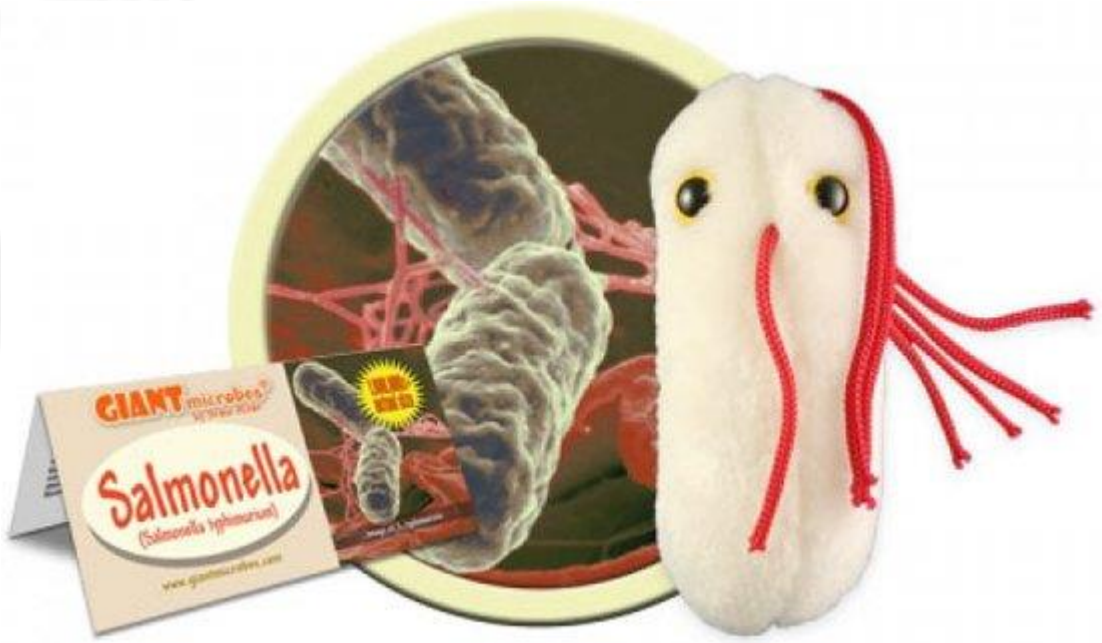
Thank you



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Case definition WHO

Suspected case of typhoid fever

Suspected case:

fever for at least 3 out of 7 consecutive days if endemic area or following travel from endemic area

OR

fever for at least 3 out of 7 consecutive days within 28 days of being in household contact with a confirmed case of typhoid case

Confirmed case:

laboratory confirmation by culture of molecular methods of S. Typhi or detection of S. Typhi DNA from anormal sterile site

relapse of typhoid fever: laboratory confirmation by culture of molecular methods of S. Typhi or detection of S. Typhi DNA from a normal sterile site within 1 month of completing an appropriate course of antimicrobial treatment and resolution of symptoms

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Case definition WHO

Chronic carriers of typhoid fever

Presumptive carrier:

evidence of shedding of Salmonella spp (positive stool culutre of PCR) of an uknown duration

Definitive carrier:

evidence of shedding of Salmonella spp (positive stool culture of PCR) at least 12 months after finishing an appropriate course of antimicrobial treatment and resolution of symptoms following a laboratory –confirmed episode of acute disease

Or

2 positive stool samples 12 months apart

Convalescent carrier:

evidence of shedding of Salmonella spp (positive stool culture of PCR) 1-12 months after finishing an appropriate course of antimicrobial

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Chronic carriers of typhoid fever

Or

2 positive stool samples 12 months apart

Convalescent carrier:

evidence of shedding of Salmonella spp (positive stool culture of PCR) 1-12 months after finishing an appropriate course of antimicrobial treatment and resolution of symptoms following a laboratory –confirmed episode of acute disease