

Department Of Biotechnology

SALMONELLA

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SALMONELLA

- Tribe – Salmonellaea
- Enteric fever, gastroenteritis, septicemia
- *Salmonella typhi* – typhoid fever
- DE Salmon – American microbiologist
- Eberth (1880) , Gaffky (1884) isolated – Eberth–Gaffky bacillus
- 2000 serotypes – two groups:
- Enteric fever, *S. Typhi*, *S. paratyphi* A and B
- Food poisoning, animal parasites – gastroenteritis, septicemia

MORPHOLOGY

- Gram-negative, non-sporing, non-capsulated 1–3 x 0.5 micron
- Fimbriae – present
- Motile, peritrichate flagella
- *S. Gallinarum* and *S. Pullorum* – non-motile

CULTURAL CHARACTERISTICS

- Aerobes and facultative anaerobes
- Colonies 2–3 mm, circular, low convex
- NA – translucent circular
- MA } NLF
- DCA } NLF

CULTURAL CHARACTERISTICS

- Wilson, Blair bismuth sulphide
- *S. Typhi* – jet black colonies, sulphite to sulphide
- *S. paratyphi* A – green colonies
- *S. paratyphi* B – black colonies
- XLD, SS agar
- Enrichment media – selenite F, tetrathionate broth

BIOCHEMICAL REACTIONS

- Glucose, mannitol, maltose – acid and gas
- Lactose, sucrose – negative
- *S. Typhi* anaerogenic
- Indole –ve, MR +ve, VP –ve, citrate +ve (*S. Typhi* – citrate –ve)
- H₂S +ve, except *S. paratyphi A*

BIOCHEMICAL CHARACTERISTICS

Table 31.1 *Biochemical characteristics of typhoid and paratyphoid bacilli*

	<i>Glucose</i>	<i>Xylose</i>	<i>d-Tartrate</i>	<i>Mucate</i>
<i>S.Typhi</i>	A	d	A	d
<i>S.Paratyphi A</i>	AG	–	–	–
<i>S.Paratyphi B</i>	AG	AG	–	AG
<i>S.Paratyphi C</i>	AG	AG	AG	–

ANTIGENIC STRUCTURE

- Flagellar antigen H
- Somatic antigen O
- Surface antigen Vi
- H antigen – heat labile
- Destroyed by boiling, alcohol
- Strongly immunogenic
- Antisera–large loose fluffy clumps

ANTIGENS

- O antigen – somatic
- Phospholipid, protein polysaccharide
- Heat and alcohol stable
- 67 O antigens
- Less immunogenic
- Antisera – compact, chalky, granular clumps

ANTIGENIC STRUCTURE

- Vi Ag – surface Ag
- Heat labile acidic polysaccharide
- Virulence – inhibits phagocytosis, resists complement activation, bacterial lysis
- *S. paratyphi C, Escherichia, Citrobacter*
- Epidemiological typing/carrier

ANTIGENIC VARIATIONS

- H-O variation: Associated with loss of flagella
phenol agar (1:800) – phenotypic change
- 901 O strain – stable, non-motile, mutant –
Craigie's tube, U tube
- Phase variation: Flagellar Ags two phases
- Phase 1 – specific/few species – a, b, c,
d, etc.
- Phase 2 – non-specific/fewer – 1, 2, etc.
- Diphasic, monophasic – *S. Typhi*

ANTIGENIC VARIATIONS

- V–W variation: V form agglutinate – Vi antiserum and not O. After subcultures ,Vi lost; W form – O antiserum; V–W both O and Vi
- S–R variation: Smooth to rough, loss of O Ag virulence, autoagglutinable – prevention – Dorsett’s egg, lyophilisation

ANTIGENIC VARIATIONS

Variation O Ag: Lysogenisation phages

S. anatum-----serotype - 3,10 e, h;1, 6

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lysogenisation - phage 15

|

S. newington-----serotype - 3,15; e h:1,6

|

lysogenisation - phage 34

S. minneapolis-----serotype -3,15, 34;
e, h: 1, 6

CLASSIFICATION

- Kauffman White scheme
- O antigen – 1, 2, 3 factors
- Factor 2 – group A
- Factor 4 – group B
- Factor 9 – group C

SALMONELLA CLASSIFICATION

Antigenic characterisation – Kauffmann White scheme

SEROGRPS	SEROTYPE	ANTIGEN		
		O	Phase I	Phase II
2-A	<i>S. para A</i>	1,2,12	a	–
4-B	<i>S. para B</i>	1,4,5,12	b	1,2
	<i>S. typhimurium</i>	1,4,5,12	l	1,2
	<i>S. chester</i>	4,5,12	e,h	e,n,x
7-C	<i>S. para C</i>	6,7(Vi)	c	1,5
	<i>S. cholera -suis</i>	6,7	c	1,5
8-C2	<i>S. muenchen</i>	6,8	d	1,2
9-D	<i>S. Typhi</i>	9,12(Vi)	d	–
	<i>S. enteritidis</i>	1,9,12	g,m	–
10-E	<i>S. Gallinarum</i>	1,9,12	–	–
	<i>S. anatum</i>	3,10	e,h	1,6

PATHOGENICITY

- Enteric fever – *S. Typhi* – typhoid
 - S. paratyphi A*
 - S. paratyphi B* paratyphoid fever
 - S. paratyphi C*
- Clinical syndromes in humans:
 - Enteric fever
 - Gastroenteritis or food poisoning
 - Septicemia

ENTERIC FEVER

- Includes typhoid fever caused by *S. Typhi*, and paratyphoid fever caused by *S. paratyphi* A, B and C
- Bretonneau – 1826 – intestinal lesions
- Budd – 1856 – transmission through excreta

ENTERIC FEVER

- ID-50-103- 106 bacilli
- Ingestion – attachment microvilli – penetration lamina propria – resist intracellular killing – mesenteric lymph nodes – multiply – thoracic duct – bloodstream (transient bacteremia – different organs – massive bacteremia – gall bladder – Payers patches

CLINICAL FEATURES

- Mild undifferentiated pyrexia – fatal fulminating disease
- Onset – headache, malaise, anorexia, coated tongue, abdominal discomfort – constipation/diarrhea
- Temperature – step ladder rise – first week, high – 7–10 days, falls by third/fourth week
- Physical signs – relative bradycardia, hepatomegaly, splenomegaly, rose spots – second/third week

COMPLICATIONS

- Intestinal perforation
- Hemorrhage
- Circulatory collapse
- Cholecystitis, arthritis, abscess
- Periosteitis, nephritis
- Venous thromboses, peripheral neuritis

EPIDEMIOLOGY

- Endemic in all parts of India
- Typhoid : paratyphoid : 10 :1
- Paratyphoid B – rare
- Carriers – convalescent, temporary, chronic

TYPHOID MARY

- Mary Mallon – New York cook – 15 years – 7 outbreaks – 200 persons affected
- Carriers – convalescent – 3 weeks–3 months – temporary >3 months – <1 year – chronic >1 year, 2–4% – fecal and urinary carriers

LABORATORY DIAGNOSIS

- Blood culture
- Clot culture
- Feces culture
- Urine culture
- Other material – bone marrow, bile

LABORATORY DIAGNOSIS

- Serology
- PCR-based tests
- Demonstration of circulating antigen
- Other tests

BLOOD CULTURE

- Positive blood culture diagnostic
- 90% positive – first week
- 75% positive – second week
- 60% positive – third week
- 5–10 ml blood collected – venipuncture aseptically, inoculated to 50–100 ml of 0.5% bile broth

BLOOD CULTURE

- After 24 hours' incubation – bile broth subcultured on MacConkey agar
- Subculture repeated – 10 days – culture negative
- Castaneda's method – biphasic medium
- **Serotyping** – slide agglutination
- **National reference centre** – CRI Kasauli

CULTURE

- Clot culture – serum – Widal test, clot – streptokinase
- Feces culture – carriers
- DCA, XLD, Wilson Blair
- Urine culture

WIDAL

- Tube agglutination test
- O agglutination – chalky granular deposit
- Felix tube
- H agglutination – fluffy, cottony deposit
- Dreyers tube



Georges Fernand Isidor Widal (1862-1929).
Demonstrated specific agglutinins in the blood of typhoid patients in 1896—
‘The Widal Reaction’.

DIAGNOSIS OF CARRIERS

- Fecal carriers – fecal culture, bile
- Urinary carriers – urine culture
- Vi antibody detection – 1:40
- SEWER SWAB TECHNIQUE – gauze pads, millipore membranes
- National Salmonella Reference Centre – Central Research Institute, Kasauli

TYPING METHODS

- BACTERIOPHAGE TYPING
 - Craigie and Yen (1937) – bacteriophage Vi II, 97 Vi phage – *S. Typhi*
 - Tracing epidemics
 - Trends and patterns epidemiology
- National phage typing centre – Lady Hardinge Medical College, New Delhi
- A–E–A–India

TYPING METHODS

- Other typing methods
- Nicolle's complementary phage typing –
Kristensen's biotyping – xylose and
arabinose
- Bacteriocin
- Antibigram

PROPHYLAXIS

- General measures – sanitation, protected drinking water
- Vaccine – TAB vaccine, *S. Typhi* 1000 million and *S. Paratyphi A* and *B* 750 million
- Dose – 0.5 ml SC 4–6 week interval

PROPHYLAXIS

- Live oral vaccine – (typhoral) – stable mutant – *S.Typhi* strain Ty2a
- Dose – one capsule orally
- Vi vaccine – injectable – purified Vi polysaccharide antigen

TREATMENT

- Rx – chloramphenicol, ampicillin, amoxycillin, streptomycin.
- Carriers – vaccine + combination therapy, cholecystectomy, pyelolithotomy, nephrectomy
- DRUG RESISTANCE – 1972 – chloramphenicol, Mexico and India

TREATMENT

- Calicut, Kerala – drug-resistant plasmids
- 1980 – multi drug resistance (MDR) – ‘R’ factor – England – 1960 – *S. typhimurium* phage 29
- Rx – fluoroquinolones (ciprofloxacin, pefloxacin, ofloxacin), third generation cephalosporins, ceftazidime, ceftriaxone
- Hospital cross-infections – neonates – septicemia

SALMONELLA GASTROENTERITIS

- Zoonotic first food – Gartner, Germany
- *S. typhimurium* – meat – 1898 – Durham, England
- *S. enteritidis* – meat from cow
- Source – poultry, meat, milk, cream, eggs, rat droppings
- Clinically – incubation period – 24 hrs/less, diarrhea, vomiting/pain, half loose stools, subsides – 2-4 days

LABORATORY DIAGNOSIS

- Feces culture, article food
- Rx – symptomatic – never antibiotics – increase period of fecal shedding of bacilli

SALMONELLA SEPTICEMIA

- *S. cholerae suis* – osteomyelitis, deep abscesses, endocarditis
- Diagnosis – blood, pus, fecal cultures
- Rx – chloramphenicol