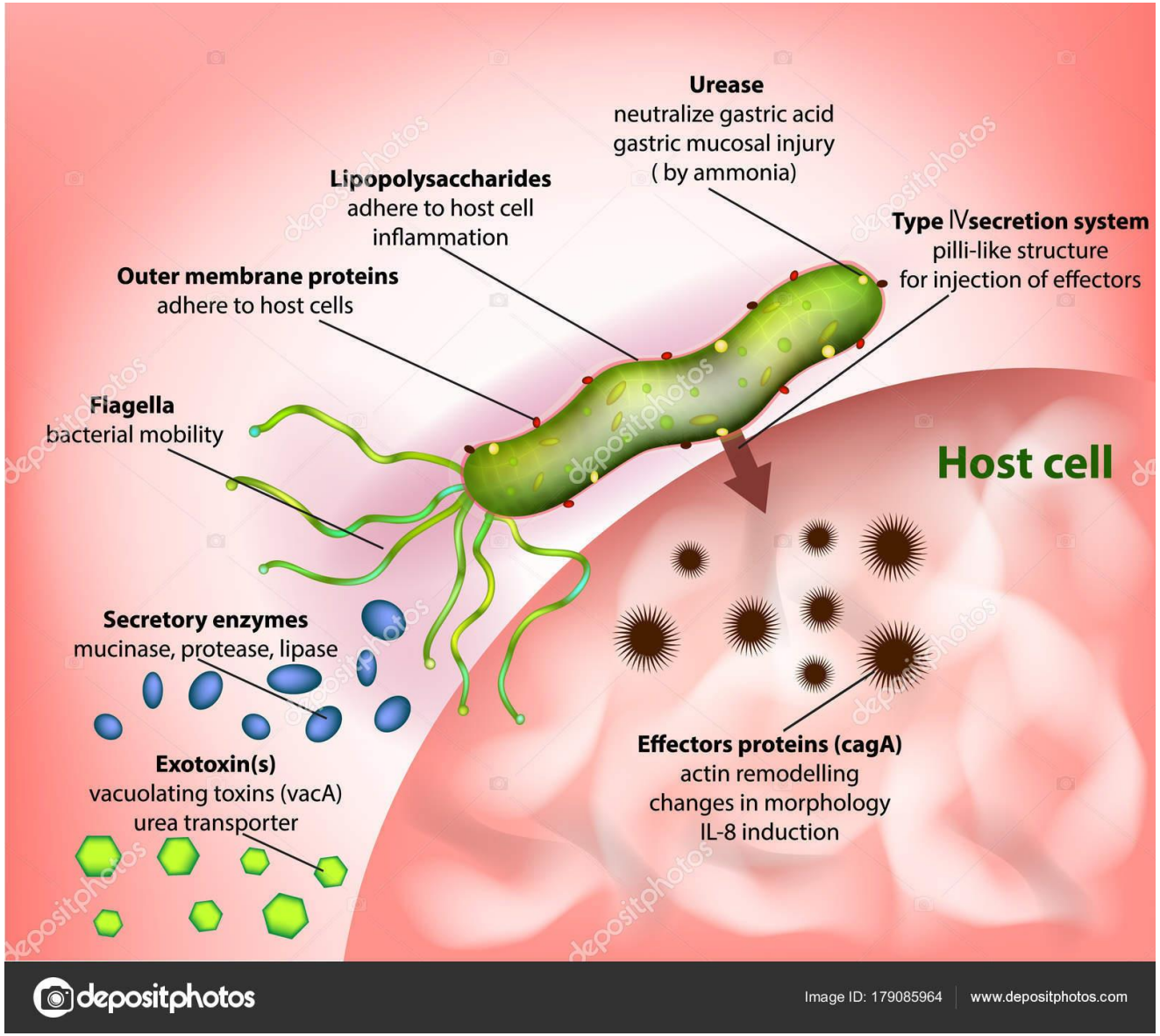
Helicobacter pylori

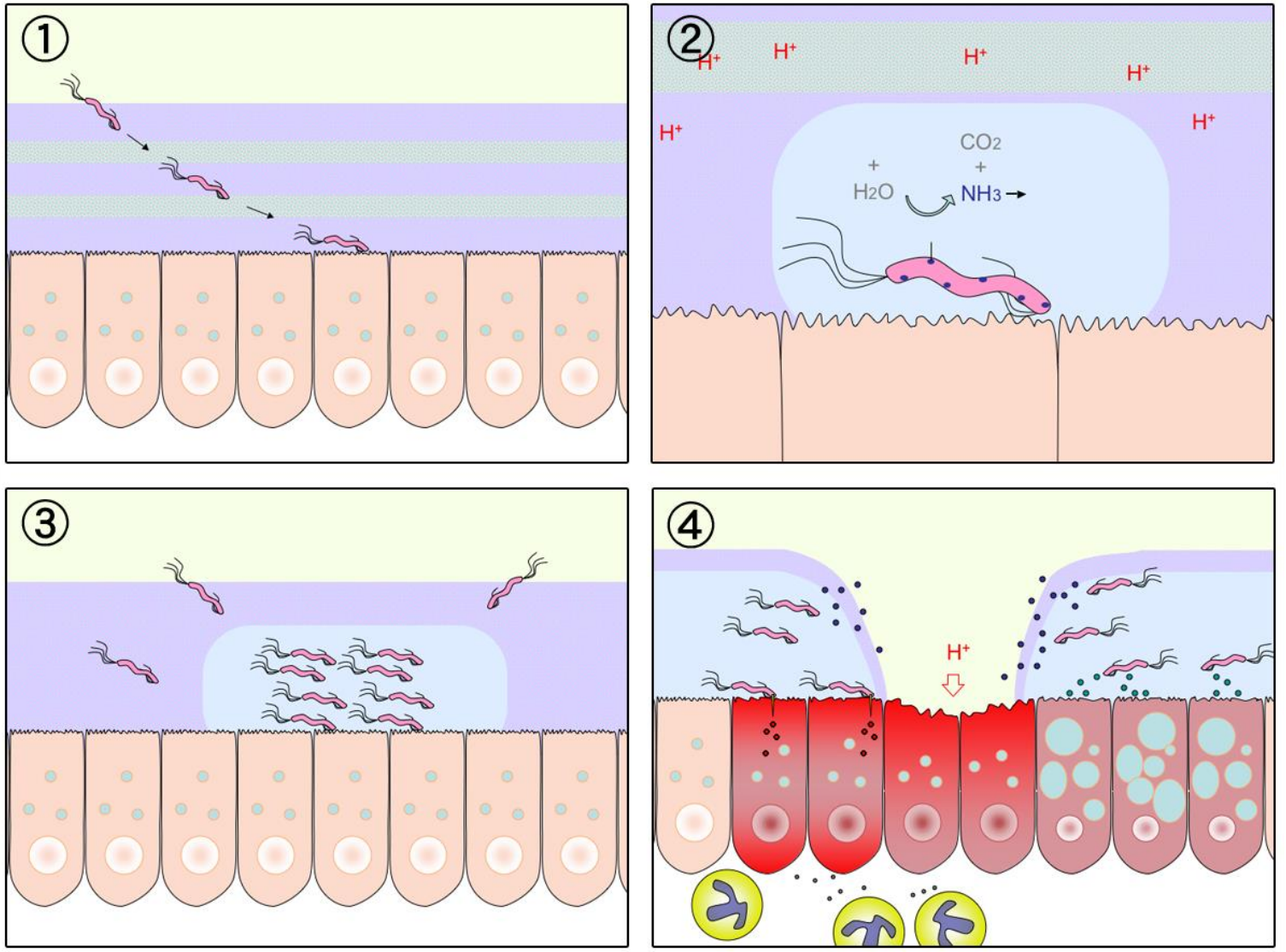
* ***Helicobacter pylori*** is a spiral (helical), a microaerophilic, gram-negative bacterium that colonizes the gastric mucosa.
* The **prevalence** of H.pylori infection in our population is estimated at **30–55%.** The prevalence increases with the age of the population. H. pylori infection is present in 90–95% of patients with duodenal ulcers and in 60–80% of patients with gastric ulcers.

* *Helicobacter pylori* is **classified** as a WHO **Group 1 Carcinogen**. However, there is no evidence that its eradication reduces the risk of stomach cancer

Virulence factors



Pathogenesis



See also: https://youtu.be/x3aZDY9Q_Qk

Etiology and epidemiology

* **motile**, curved microbe with **flagellum**
* lives in the mucus **the gastric mucosa**
* high **urease** production
* a purely **human pathogen**, has not been detected in animals or soil

about 50% of the population is estimated to be infected; the incidence is lower in developed countries

* **transmission** – **orofecal** or **oro-oral**; direct and indirect transmission from person to person is possible (e.g. contaminated food, dishes)

most infections are acquired in childhood - the most common is direct transmission from an infected mother; but there are also new infections in adulthood, especially people with impaired immunity

Symptoms

* Helicobacter colonizes mainly the **mucosa** of the **antrum** of the stomach, later the body, but also the cardia. The settlement is focal, not diffuse; therefore, a larger number of endoscopic biopsies is required for capture. Colonization of the **gastroduodenal mucosa** is accompanied by the development of **chronic gastritis**, which represents a **heterogeneous group of inflammatory processes** of various etiologies. Prolonged chronic gastritis caused by *H. pylori* can lead to mucosal atrophy and **intestinal metaplasia**, the most common precursor of intestinal gastric adenocarcinoma.
H. pylori infection has a causal relationship to the **peptic ulcer of the gastroduodenum**.
* **Disruption of the mucosal barrier** defense mechanisms (surfactant, mucus, basement membrane of gastric epithelial cells) by bacteria is followed by the **release of inflammatory metabolites of epithelial cells** and is one of the most important factors in the pathogenesis of chronic gastritis and peptic ulcers. It is not known to what extent the degree of inflammation is affected by the characteristics of the host (genetic factors) or pathogen (phenotype, genotypes).
* Eradication of *H. pylori* infection leads to **healing of the ulcer**.

Helicobacter extraintestinal diseases and syndromes

SIGNIFICANT

*** larynx disorders**

benign: chronic laryngitis (incidence of infection in almost 46% of patients in the research), vocal cord polyps (singing nodule)

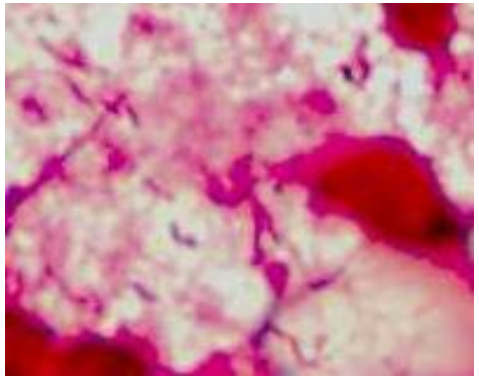
malignant: laryngeal tumors (incidence of infection in 46% of patients in the research)

Also documented

* autoimmune disease (idiopathic trombocytopenic purpura, autoimmune thyreoiditis)
* skin condition (acne, rosacea, idiopathic chronic urticaria)
* endocrine disorders (thyreopatia)
* neurological disorders (migraine)
* hepatobiliary disease
* cardiac (ICHS), vascular (Raynaudova choroba)
* recurrent dyspepsia/discomfort
* iron-deficiency anemia
* growth disorders

Diagnosis

- A stool polymerase chain reaction (**PCR**) test, stool **antigen test**
- Serological examination of antibodies against Helicobacter pylori by ELISA method (IgG, IgA)
- Endoscopically obtained biopsy specimens can be using Gram staining (see also the fig. below) tested by rapid urease test, histologically, or by culture (Skirowa's soil) – **identification MALDI TOF**
- 13C-labeled urea breath test
- Serological detection of preneoplastic markers may be useful in preventing gastric cancer



Gram stained bioptate with Gramnegative spiral (or „flying bird“ shaped) bacteria

Urease test
Positive on the right (red colour)

Treatment

* The gold standard of eradication is a **triple combination of drugs** given for 7 days:

omeprazole + amoxicillin + clarithromycin (or any other macrolide)

* If the patient has a penicillin allergy: omeprazole + metronidazole + clarithromycin

* In recent years, there has been a **decline in the successful eradication** of H.p. after standard triple combination therapy with **increased resistance to antibiotics**, especially clarithromycin. Where there is increasing resistance to clarithromycin, bismuth quadruterium (bismuth) is recommended. Probiotics can reduce the incidence of side effects of standard eradication. The most successful eradication treatment is now the subject of numerous studies.

Campylobacteriosis

Etiology, epidemiology

- most frequent worldwide diarrheal infection
- **hemorrrhagic enterocolitis** – immunocompetent patients
- extraintestinal – immunocompromised
- Gramnegative rods, microaerophilic, special culture media, hihger temperature (42C), *Campylobacter jejuni*, *C. coli* and others
- zoonosis – commensal in the intestine of wild and domestic animals, source – ingestion of contaminated food (e.g. chicken, pork)

Campylobacteriosis

etiology, epidemiology

• incubation period – 2-7 days

• **hemorrrhagic enterocolitis** – immunocompetent patients

• extraintestinal – immunocompromised

*** pathogenesis:** invasive, production of toxins, found – jejunum, ileum, colon

*** symptoms:** from secretory diarrhea to severe illness, most common – hemorrhagic enterocolitis – mucuc and blood, high fever, rarely extraintestinal – sepsis or localized (e.g. meningitis)

Campylobacteriosis

Pathogenic Campylobacter species

Worldwide, pathogenic *Campylobacter* species are responsible for the cause of over **400–500 million infections cases each year**. Pathogenic *Campylobacter* species known to be implicated in human infections includes *C. jejuni*, *C. concisus*, *C. rectus*, *C. hyointestinalis*, *C. insulaenigrae*, *C. sputorum*, *C. helveticus*, *C. lari*, *C. fetus*, *C. mucosalis*, *C. coli*, *C. upsaliensis* and *C. ureolyticus*. These pathogenic *Campylobacter* species are grouped into **major human enteric pathogens** (*C. jejuni*, *C. jejuni* subsp. *jejuni (Cjj)*, *C. jejuni* subsp. *doyley* (*Cjd*), *C. coli* and *C. fetus*); **minor pathogens** (*C. concisus*, *C. upsaliensis, C. lari* and *C. hyointestinalis*) and **major veterinary pathogens** (*C. fetus* subsp. *venerealis* (*Cfv*) and *C. fetus* subsp. *fetus* (*Cff*)).

Ref: Igwaran A. Human campylobacteriosis: A public health concern of global importance, *2019*

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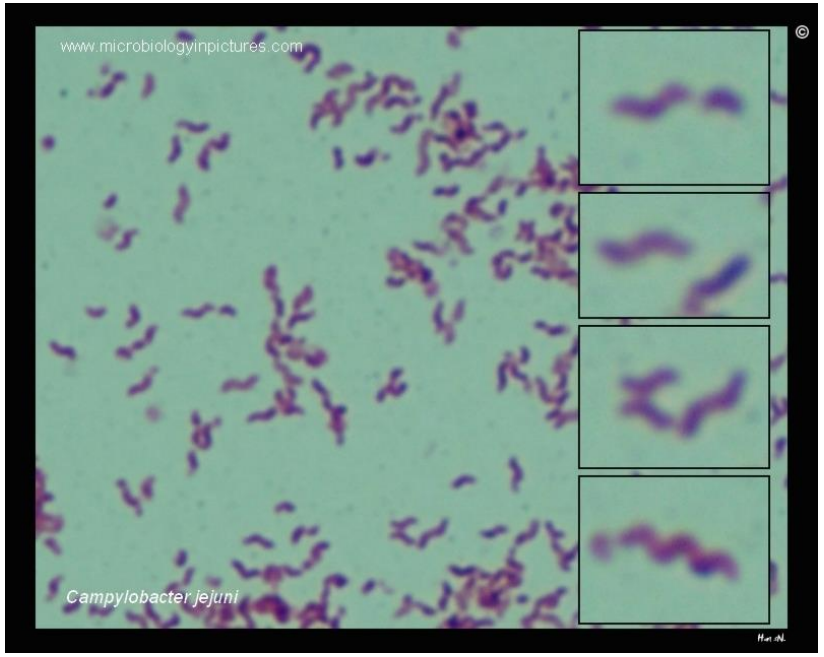
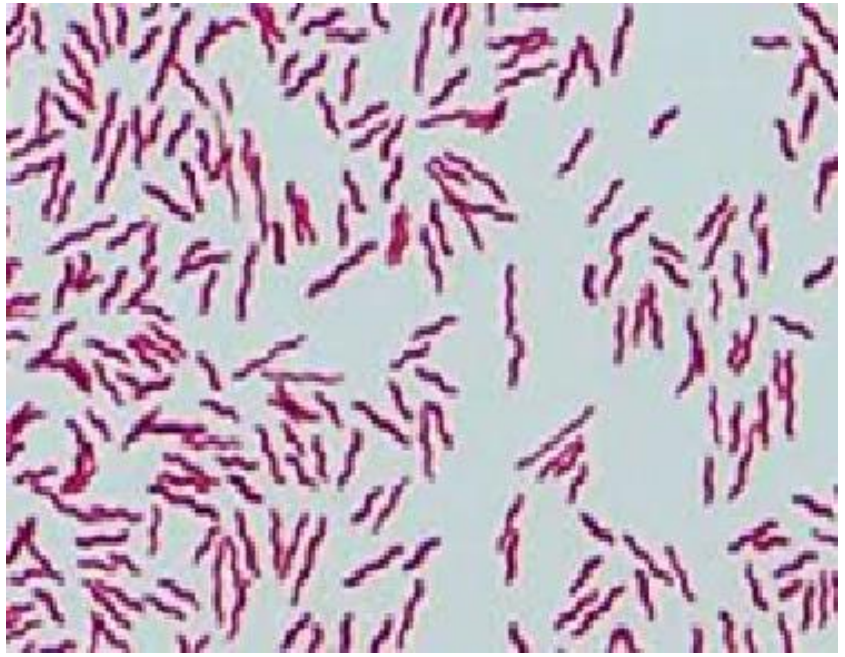
Campylobacteriosis

- ***C. jejuni*** is a motile, microaerophilic, zoonotic, thermophilic bacterial considered as the leading cause of worldwide foodborne bacterial gastroenteritis .
- It's a member of the genus *Campylobacter* with polar flagella and helical morphology that is used for movement through viscous solutions including the mucus layer of the gastrointestinal tract





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Campylobacteriosis

- ***Campylobacter coli*** is an S-shaped curved cell measuring about 0.2–0.5 micrometers long with a single flagellum. It's very similar to *C. jejuni*; and both bacteria cause inflammation of the intestine and diarrhea in humans.
- ***C. fetus*** is a curved cell, fastidious motile bacterial that majorly cause septic abortion in farm animals. *C. fetus* can cause infection in human and its infection can be acquired through direct contact with animals, through consumption of undercooked contaminated meat or through ingesting food or water contaminated by animal faeces. **Reported to be associated with human infection such as bacteremia. It's an opportunistic human pathogen that largely infects immunecompromised patients.** Some of the major reported symptoms of *C. fetus* infections include **endocarditis, meningitis, septicemia, septic arthritis, peritonitis and cellulitis,** but infections are rare.

Pathogenicity of *Campylobacter* species

- *Campylobacter* **pathogenicity is based on the virulence factors** (Larson et al., 2008) and these **virulence factors are multi-factorial** in nature and the ability of these bacteria to survival and resist physiological stress also contributes to its pathogenicity (Casabonne et al., 2016; Ketley, 1995). The various virulence related mechanisms displayed by *Campylobacter* species includes **invasive properties**, oxidative stress defence, **toxin production**, iron acquisition and its ability to remain viable but non-culturable state (Bhavsar and Kapadnis, 2006). *Campylobacter* **invasion**, **adherence** and colonization also add to the pathogenicity of these groups of bacteria (Backert et al., 2013). **Other virulence factors** of *Campylobacter* include; **secretion of some sets of proteins**, **translocation capabilities** and **flagella-mediated motility** (Biswas et al., 2011).

Motility and flagella

- **Motility is important for *Campylobacter* survival under diverse chemotactic conditions it comes across in the gastrointestinal tract** (Jagannathan and Penn, 2005). In some *Campylobacter* species, the motility system with the **flagella involves a chemosensory system** that steers flagella movement depending on the environmental conditions where these bacteria are found. *Campylobacter* **chemotaxis** and **flagellin** are the two important **lead these bacteria to its colonization site** and also **help in invading** the host cell (van Vliet and Ketley, 2001).
- **Chemotaxis** - is a method or system by which **motile bacteria sense and move to the direction of more favourable conditions** and several pathogenic bacteria uses this practice to invade their hosts.

Adhesion

- **Colonisation mediated by some adhesins on the bacterial surface (Jin et al., 2001).** *Campylobacter* adhesion virulence factors includes **outer membrane protein**, ***Campylobacte*r adhesion protein A**, **phospholipase A, lipoprotein, periplasmic binding protein, fibronectin-like protein A and Type IV secretion system** (Bolton, 2015). *Campylobacter* adhering to fibronectin F is another important *Campylobacter* **virulence factor that enables these bacteria to bind to fibronectin** which **promotes the bacterium-host cell interactions and colonization** (Konkel et al., 2010). Other virulence genes in *Campylobacter* species reported to be linked with human infections responsible for **expression of colonization and adherence** (Datta et al., 2003).

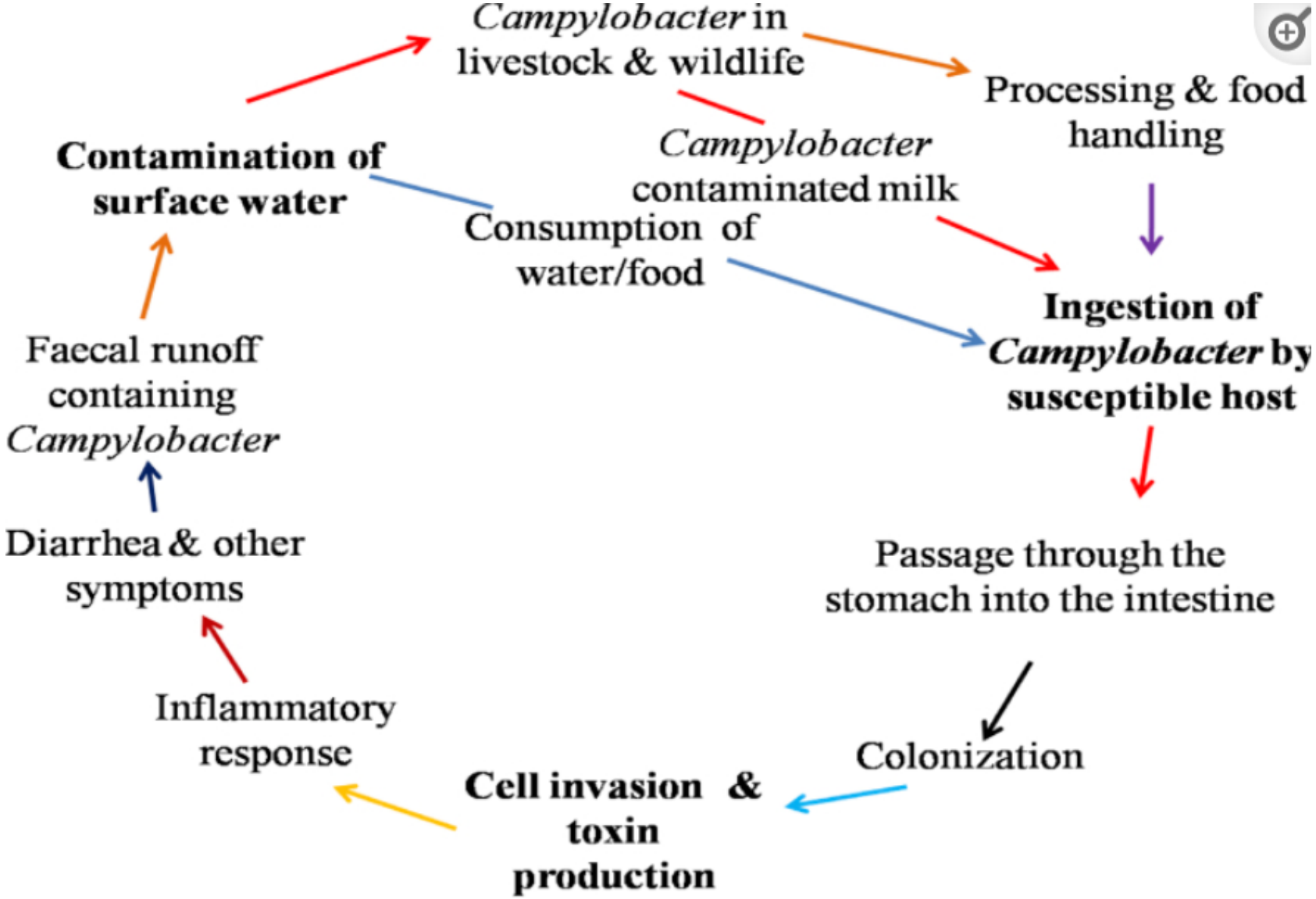
Toxin production

- *Campylobacter* produce different type of **cytotoxins** and **cytolethal distending toxin (CDT)** is one of these toxins **(Schulze et al., 1998).** CDT is a **tripartite toxin that is made up of three subunits encoded. Activity is determined by these three *cdt* cluster genes (Martinez et al., 2006).** The *cdtA* and *C* genes are **heterodimeric toxin subunits** responsible for **toxin binding and internalization of the host cell while *cdtB* is the subunit which encodes for the toxic/active components of the toxin (Abuoun et al., 2005). Cytolethal distending toxins induce diarrhea in both humans and animals by intrusive with the division of cells in the intestinal crypts (Carvalho et al., 2013).**

Invasion

- **Invasion is another virulence mechanism in *Campylobacter* that is carried out by the flagella** which also function as an export apparatus in the secretion of non-flagella proteins during host invasion **(Poly and Guerry, 2008). Flagella secretion system is vital for invasion and colonisation (Konkel et al., 2004). The secretion of invasion antigens and invasion protein B (*ciaB*) are also important virulence proteins** synthesized by *Campylobacter* species which **help in the epithelial cells invasion and adhesion of the host gastrointestinal tract (Casabonne et al., 2016). The periplasmic protein HtrA responsible for full binding to the epithelial cells.**

Campylobacter infections



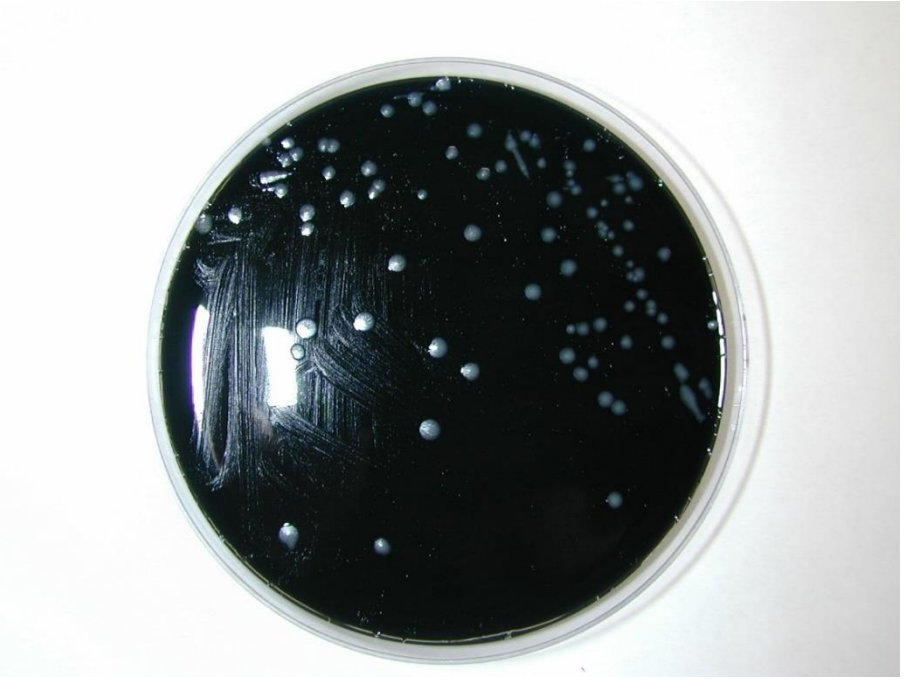
Extraintestinal infections

* Are infections **outside the intestines** but symptoms are associated with a problem within the intestine (Hernandez and Green, 2006). Extragastrointestinal infections reported to be associated with *Campylobacter* infections includes **reactive arthritis**, **Guillain-Barré syndrome -** GBS (Kuwabara and Yuki, 2013), **bacteremia, septicaemia** (Man, 2011), **septic arthritis, endocarditis, neonatal sepsis, osteomyelitis, and meningitis** (Allos, 2001).

Campylobacteriosis

Diagnosis: stool culture, special media and atmosphere, extraintestinal – blood culture and other (CSF…), **identification MALDI TOF**

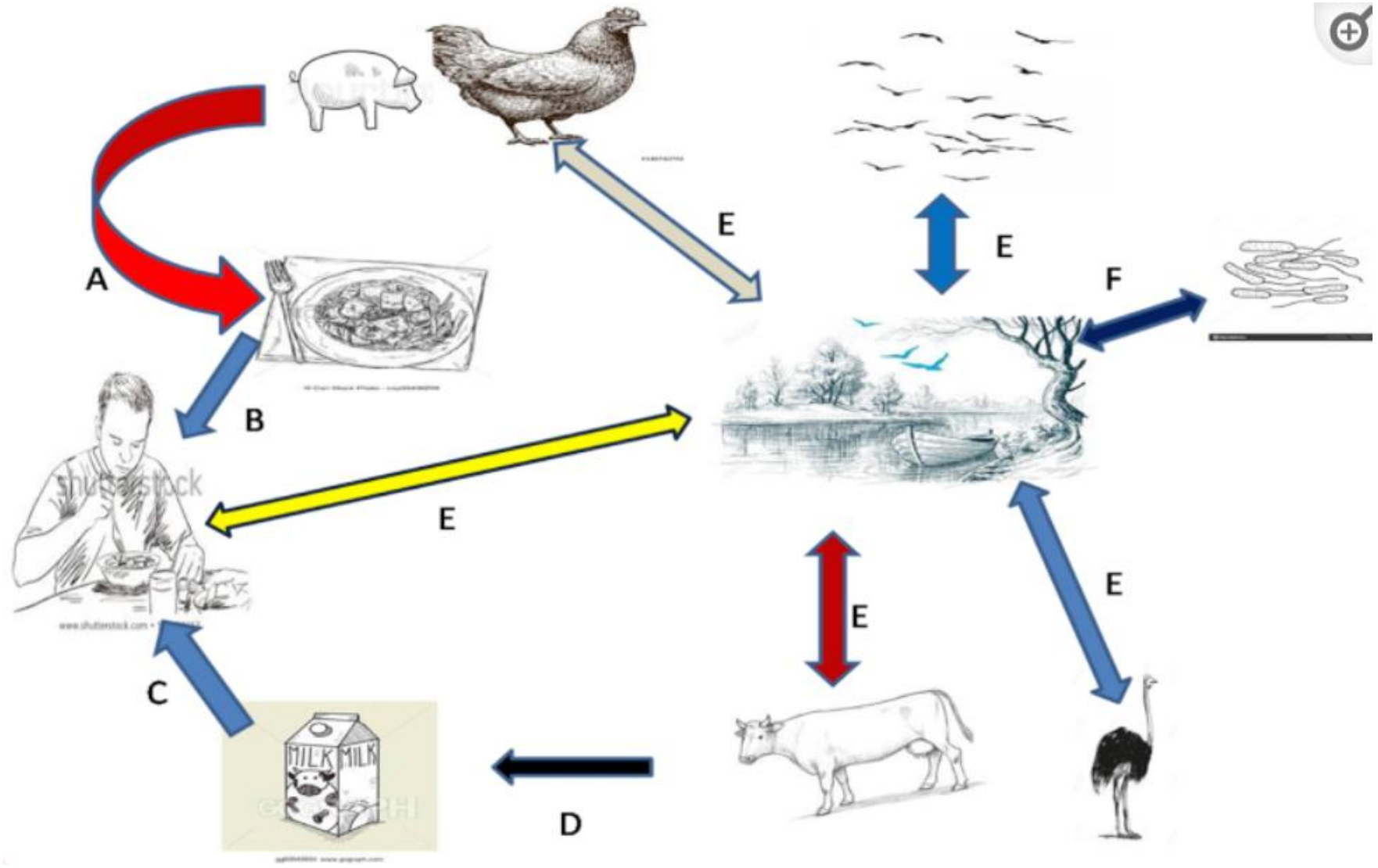
Treatment: mild or moderate cases – rehydratation, diet, adsorbent, probiotics, severe diarrhea and systemic inf. – **macrolides,** fluoroquinolones (systemic inf. – weeks depending on susceptibility)



Charcoal-based selective media (CSM)

- Specimen – swab, stool and transport media
- e.g. Charcoal-based selective media (CSM) for isolation Campylobacter spp. from fecal specimes – small whitish or greyish colonies
- microaerophilic atmosphere at 42C, 48h
- microscopy – curved Gramnegative rods
- phenotypical identification – oxidase positive, mass spectrometry (MALDI)

Transmission routes of *Campylobacter* infection



Conclusion

Worldwide, outbreaks of campylobacteriosis have been increasing and the major routes of transmission of these bacteria to human is generally believed to be through consumption of contaminated foods. The development of rapid Kits for *Campylobacter* detection and quantification in foods from animal origin will be essential for the prevention of *Campylobacter* infections. *Campylobacter* infections are majorly treated with antibiotics and the actions of these antibiotics have been compromised and this call for the development of new vaccines that will help to control the regular use of antibiotics in animal husbandry. In addition, regular domestic hygiene will also help to prevent *Campylobacter* infections. The production of new and effective antibiotic for better treatment of campylobacteriosis will as well help in the reduction of antibiotic-resistant *Campylobacter* strain and the spread of antibiotics resistant genes.

Non-sporeforming anaerobic bacteria

Grampositive cocci: ***Peptostreptococcus, Peptococcus spp.*** **– inhabitant of oral cavity, GIT, skin,** infection – when they spread from the normal sites (e.g. from upper respiratory tract to sinuses – **sinusitis**, from genitourinary tract – **endometritis, pelvic abscesses)**, **therapy** – **usually susceptible to penicillin**

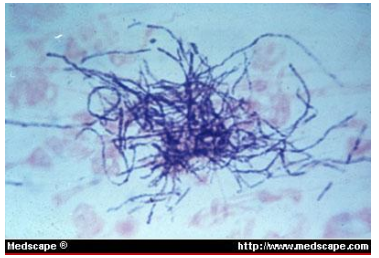
Grampositive rods: ***Actinomyces spp.*** **–** *e.g.* ***A. israelii,*** **inhabits mucosal surfaces –upper respiratory tract and female genitourinary tract**, **grow slowly** (cca 2 weeks) on culture media, microscopy **gram-positive rods and filaments**, low virulence – causes **endogenous disease** only when normal mucosal barriers are disrupted by trauma or surgery. **Clinical infections** – **cervicofacial actinomycosis** (after poor oral hygiene, after invasive dental procedures), thoracic actinomycosis (after aspiration), **abdominal and pelvic inf**. after **GIT surgery** or as **primary inf. n women with intrauterine device**, **treatment** – tissue debridement and penicillin.

Other grampositive rods: ***Propionibacterium acnes*** – after digestion by PMN in sebaceous glands enzymes like lipases are released and cause inflammation in teenagers, can causes also opportunistic infections (e.g. infection of artifical heart valves). ***Lactobacillus*** **spp**. – part of normal flora of the mouth, stomach, intestines, and genitourinary tract (vagina – normal flora with probiotic effect, lowering pH)

Diagnosis of actinomycosis

Dermatologist.com

Sample collection: pus



Microscopy preparation, Gram-positive rods and filaments
PCR detection

Culture after 14days of cultivation

Microscopy prepation, Gram-positive rods and filaments

Identification (mass spectrometry)

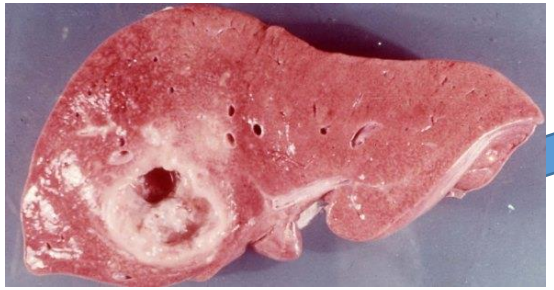
Identification by PCR

Diagnosis: cervicofacial actinomyosis (lesion – actinomycetoma)

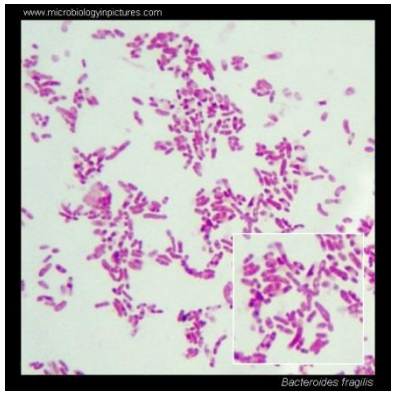
Non-sporeforming anaerobic bacteria

Gram-negative cocci: ***Veilonella*** **spp. – microbiota of GIT – can cause endocarditis, sepsis, drug of choice - penicillin**
Gram-negative rods: ***Bacteroides*** **spp.(50 species)** – LPS lower endotoxic activity**,**
• Colonize the **upper respiratory tract, GIT, genitourinary tract**
• Most significant pathogen - ***Bacteroides fragilis*** *,* **resides in GIT**, causing **abdominal infections** (e.g. liver absceses) and bacteremia after disruption of natural barriers by diagnostic or surgical procedures, **pleomorphic gram-negative rods**, capsule – adhesive and antiphagocytic properties, **laboratory diagnosis** – culture, treatment – usually produce beta-lactamases, **drug of choice – metronidazole,** carbapenems.
• ***Fusobacterium necrophorum –*** *gramnegative* ***rods or filaments, inf.*** *cecrrotizing pneumonia, lung abscesses,* ***Plaut-Vincent tonsilitis – necrotizing tonsilitis,*** *drug of choice -* ***metronidazole***
• ***Prevotella melaninogenica –*** resides in GIT, can cause abscesses in various localization (mainly respirátory tract, sinusitis), drug of choice - **metronidazole**

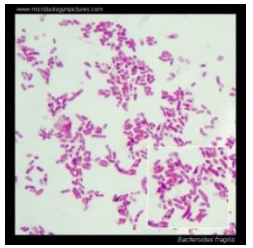
Diagnosis of liver abscess infection



Sample collection: pus



Microscopy preparation, negative pleomorphic rods
PCR detection



Microscopy preparation, negative pleomorphic rods

Culture

Identification (mass spectrometry)

Identification by PCR

Diagnosis: liver abscess caused by *B. fragilis*

Diagnosis of anaerobic infections

Clinical signs include:

- foul-smelling discharge (becuase of the end product of anaerobic metabolism – short-chain fatty-acids)
- infection in proximity to a mucosal surface (anaerobes are part of the normal flora)
- gas in tissue (production of CO_2 and H_2)
- negative aerobic cultures
- positive anaerobic culture, **identification MALDI TOF**

The place of molecular genetic methods in the diagnostics of anaerobic bacteria (Nagy et al., 2006, Acta Microbiol Immunol Hung.)

- diverse group of bacteria
- specimens yielding anaerobic bacteria commonly contain several organisms and often very complex mixtures of aerobic and anaerobic bacteria, considerable time may elapse before the final report.
- Species definition based on phenotypic features is often time-consuming and is not always easy to carry out.
- Molecular genetic methods may help in the everyday clinical microbiological practice (such as 16S rRNA PCR-RFLP profile determination), which can help to distinguish species
- Some anaerobic bacteria are extremely slow growing or not cultivable at all but detectable by molecular methods which also demonstrated the spread of specific resistance genes among the most important anaerobic bacteria.
- Molecular methods (a search for toxin genes and ribotyping) may promote a better understanding of the pathogenic features of some anaerobic infections, such as the nosocomial diarrhoea caused by *C. difficile* and its spread in the hospital environment and the community.