Thesis

Antibiotic-associated diarrhoea and *Klebsiella oxytoca*

Submitted by

Florian Ulbl

Mat.Nr:0212187

for the degree of

Dr. med. univ.

at the

Medical University of Graz

Institute of Experimental and Clinical Pharmacology

1st Supervisor

Ao. Univ. Prof. Dr. Irmgard Lippe

2nd Supervisor

Ao. Univ. Prof. Dr. Christoph Högenauer

Graz, …….. (Florian Ulbl)

Eidesstattliche Erklärung

Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, andere als die angegebenen Quellen nicht verwendet habe und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Graz, am …… *Unterschrift*

Acknowledgement

I am particularly grateful to my supervisors Ao. Univ. Prof. Dr. Irmgard Lippe and Ao. Univ. Prof. Dr. Christoph Högenauer who have made a lot of efforts to support me with this work. Also I am much obliged to Mag. Martina Joainig who has spend a lot of time and commitment to give me an understanding of laboratory work.

Abstract

Antibiotic-associated diarrhoea (AAD) is a common side effect of treatment with antibiotic agents. Around 10 to 20% of cases are caused by pathogenic bacterial overgrowth.

Antibiotic-associated hemorrhagic colitis (AAHC) first described in 1978 was recently found to be caused by *Klebsiella oxytoca*. Not much is know yet about the underlying pathophysiological mechanisms. But since other forms of toxin-induced colitis resemble findings of AAHC it has been suggested that the disease is cytotoxin mediated.
We have made efforts to gather information on demographic and clinical characteristics from patients with AAHC. Data from 20 cases diagnosed at the Medical University of Graz were collected and retrospectively analysed. To confirm the diagnosis, made on the basis of clinical history patients underwent diagnostic colonoscopy. Stools were examined and tested negative for bacteria commonly causing AAD. Consistent with typical findings on endoscopy and the absence of *C. difficile* infection, the disease was classified as antibiotic-associated haemorrhagic colitis. *Klebsiella* strains from stool samples were cultured with the use of MacConkey agar plates and isolated. In order to substantiate the hypothesis that pathogenic *Klebsiella* strains produce toxins responsible for the cytotoxic effects seen in AAHC further microbiological and biochemical analyses - as the API 20E test for identifying colonies and the MTT test for assessment of cytotoxic activity as well as a PCR – were subsequently conducted. The assumption that findings in AAHC were toxin induced could be confirmed with further evidence. Typically, patients suffering AAHC are young and otherwise healthy. Although *K. oxytoca* does not represent a frequent cause of diarrhoea incidence was higher than for some other common enteric pathogens. Since symptoms resolve spontaneously after cessation of antibiotics the disease might be underdiagnosed and the actual prevalence therefore is suspected to be higher outside the hospital setting.

Clostridium difficile-associated diarrhoea (CDAD) is an own entity of antibiotic-associated diarrhoea. With an estimated incidence of 110 per 100.000 admissions CDAD is the most common nosocomial infection in many European hospitals. It is exceeding diseases due to methicillin-resistant *Staphylococcus aureus* (MRSA) and can involve a mortality rate of 20%. The disease therefore is of increasing relevance for health care institutions. With regard to the major impact of *C. difficile* on public health questions it has received special attention in the work at hand. Current literature has been reviewed and discussed to deliver compressive information on this emerging issue.

Kurzdarstellung

Die Antibiotika- assoziierte Diarrhö (AAD) ist eine häufige Nebenwirkung bei der Behandlung mit Antibiotika. Ungefähr 10% bis 20% der Fälle sind auf die Fehlbesiedelung des Darmes mit pathogenen Keimen zurückzuführen.

Die Antibiotika- assoziierte hemorrhagische Kolitis (AAHC) wurde im Jahr 1978 beschrieben. Erst im Jahr 2006 konnte das Bakterium *Klebsiella oxytoca* als auslösendes Agens identifiziert werden. Doch über die zugrunde liegenden pathophysiologischen Mechanismen der Erkrankung ist bis heute wenig bekannt. Die Vermutung wurde nahegelegt, dass die AAHC durch Zytotoxine mediiert wird. Wir haben Anstrengungen unternommen, Informationen über demographische und klinische Charakteristika von Patienten mit AAHC zu gewinnen. Daten von 20 AAHC-Fällen wurden an der Medizinischen Universität Graz gesammelt. Die auf Basis der Anamnese gestellte Diagnose konnte durch die Befunde einer Koloskopie bestätigt werden. Eine Infektion mit *C. difficile* oder anderen typischen Erregern der AAD wurde ausgeschlossen. *Klebsiella oxytoca* wurden aus dem Stuhl isoliert und kultiviert. Um das zytotoxische Potential der pathogenen Stämme zu evaluieren, wurden spezifische Analysen durchgeführt. So wurde sowohl der API 20E-Test als auch eine PCR für die Identifizierung der Bakterienstämme eingesetzt. Ein MTT-Test wurde herangezogen um die zytotoxische Aktivität der Kulturüberstände zu beurteilen. Die Vermutung, dass die AAHC Toxin induziert sei, konnte durch weitere Evidenz untermauert werden. Typischerweise sind die Betroffenen jung, und ansonsten gesund. Obwohl *K. oxytoca* keine häufige Ursache einer Diarrhö darstellt, war die Inzidenz höher als für einige andere bekanntere Erreger der Enteritis. Da die Symptome nach Beendigung der Antibiotikaeinnahme wieder spontan zurücktreten, könnte die Erkrankung allgemein unterdiagnostiziert sein. Die wahre Prävalenz wird daher im ambulanten Bereich als durchaus höher eingeschätzt.

Die *Clostridium difficile*-assoziierte Diarrhö (CDAD) ist eine Sub-Entität der Antibiotika-assoziierten Diarrhö. Mit einer geschätzten Inzidenz von 110 pro 100.000 Krankenhauseinweisungen ist die CDAD eine der häufigsten nosokomialen Infektionen im europäischen Raum. Damit übertrifft die CDAD bereits Infektionen durch *Methicillin-resistente Staphylokokken* (MRSA). Die Erkrankung kann mit einer Mortalitätsrate von 20% einhergehen. Die CDAD ist also von zunehmend wichtigerer Bedeutung für die Gesundheitsberufe. Um dieser Bedeutung gerecht zu werden, wurde in der vorliegenden Arbeit eine besondere Gewichtung auf das Bakterium *C. difficile* gelegt. Anhand rezenter Literatur wurde versucht, das Thema detailliert und aktuell darzustellen.

List of Tables

List of Figures

Introduction

Antibiotic-associated diarrhoea (AAD) is a common side effect of treatment with antibiotic agents.[69]Depending on the antibiotic used, up to 25% of patients experience such unpleasant bowel conditions.[39 40 74]The clinical spectrum of AAD varies from mild diarrhoea to life threatening colitis if complications occur.[2]

The aim of this diploma thesis is to comment on the 10 to 20% of AAD cases caused by pathogenic bacterial overgrowth. A discussion of current literature given in the last chapter is complemented by the practical part of this work.

Above all, diseases of increasing relevance, namely *Clostridium difficile*-associated diarrhoea (CDAD) such as pseudomembraneous colitis (PMC) will be elucidated. Also a brief overview will be given about physiologic bowel flora and common hypotheses of non-infectious antibiotic-associated diarrhoea prior to further explanations about infectious causes of AAD.

The work places a particular emphasis on the bacterium *Klebsiella oxytoca* which was recently found to be causally responsible for the antibiotic-associated hemorrhagic colitis (AAHC)[41] seen as an entity of antibiotic-associated-colitis (AAC).

The practical work aims to substantiate the hypothesis that pathogenic *Klebsiella* strains produce toxins responsible for the cytotoxic effects seen in AAHC.[41]
Therefore clinical and demographic data of AAHC-patients treated at the Medical University of Graz in the period of 1996 and 2008 were collected and retrospectively analysed.
Patients were tested for *Klebsiella spp.* and isolates were further tested cytotoxin production.

Backgrounds of AAD

Physiolgic bowel flora

The human colon contains a high amount of physiologic bacteria.[31] The normal colonisation of the colon is about 10^{11-12} bacteria per ml of colon content whereas the proximal small bowel (from the stomach to the upper ileum) holds a considerably lower quantity of physiologic bacteria. The jejunum contains 10^4 and the lower ileum about 10^9 bacteria per ml stool. Numerous factors contribute to the stability of the bowel flora.[78]

In healthy subjects the microorganisms in the upper intestine are mainly represented by Gram-positive anaerobe strains.[70 85] They enter the digestive tract via the oropharynx and are passed through with each meal. The whole intestine performs a complex and well coordinated movement that serves mixing, transport and clearance of the nutrient swallowed.[24] If the bowel transit is accelerated, intestinal mash is transported faster and allows a higher quantity of aerobe bacteria to settle in more distal bowel segments, as far down as the colon. But in the physiologic microflora of the colon, strictly anaerobes such as *Bacterioides*, anaerobe *Streptococcus* and *Clostridium*, outnumber aerobe bacteria by a 1000 fold! As a result aerobe, often aberrant types (e.g.: *Klebsiella, Enterobacter* and *Proteus*) increase in number and can cause disease.

Control mechanisms of a healthy individual that influence microflora and potentially reduce pathogenic bacteria include a sufficient concentration of gastric acid in the stomach and antibacterial bile acids. Ultimately the physiologic microflora produces antibacterial substances itself to defend against competitors.[33]

Antibiotic-associated diarrhoea (AAD)

AAD is defined as the passing of three or more loose stools per day occurring after application of an antibiotic within a few hours or a period of not more than two months after cessation of antibiotics.[69]

Antibiotics are commonly causing digestive dysfunction. Variable numbers are stated in literature, differing between 2-7%[69] and 15-25%[74] depending on the substance used.

Table 1: Antibiotics suspected to cause diarrhoea

Frequent (up to 20%)	Occasional	Rare
Ampicillin/Amoxicillin	Macrolides	Aminoglycosides
Clindamycin	Penicillin	Fluorochinolon*
Cephalosporin	Co-Trimoxazol	Bacitracin
	Lincomycin	Rifampicin
	Tetracyclin	Isonicid
		Metronidazole
		Vancomycin

*Fluorochinolons have also been mentioned as high risk drugs by Prof. Högenauer C.
Data from references [74]

Antibiotics with a high incidence of more than 20% are penicillin derivatives like ampicillin/amoxicillin as well as clindamycin and cephalosprines.[74] Additionally mentioned as high risk drugs are chinolones/fluorochinolones of the third and fourth generation. (personal communication with Prof. Högenauer C)
(see Table 1)

AAD without colitis

The predominant number of AAD cases are benign, self limiting “dysfunctions” without signs of colitis, well known by medical professionals and accepted without further differential diagnostic considerations.[10] [74] (also see Figure 1) The pathophysiological changes in antimicrobial treated individuals leading to diarrhoea are not yet fully understood.

Common hypotheses of AAD without colitis will be discussed:

Carbohydrate metabolism

Normal gut flora metabolises not absorbable carbohydrates to absorbable short-chained fatty acids (SCFA).[69] Between 20-50 g of carbohydrates are converted into 200-700 mmol SCFAs daily. As mentioned above, antibiotic treatment can lead to changes in intestinal bacterial flora with a decline in anaerobic species and a possible decrease of the fermentation rate of 70% of unabsorbable substrates.[74] Not metabolised carbohydrates remaining in intestinal mash operate as osmotic substances attracting water. While the volume of the intestinal mash is augmented by the attracted water, distension of the bowel results in accelerated stool transit

and thus leads to diarrhoea with volumes usually less then 500g per day.[69] Decreased fermentation can be assumed by a low H_2 excretion in breath.[74]

7α-dehydroxylase-activity

Primary bile acids (chendeoxychol acids) are dehydroxylated into secondary acids by Gram-positive anaerobe bacteria in the colon. Impairment of anaerobes results in an increase of primary bile acids causing intestinal secretion.[69] It is not clear whether this mechanism plays a significant role or not.

Suspected dysfunctions of carbohydrate or bile acids metabolism can be verified by analyses of stool. However, these changes are usually transient and self-limiting and are therefore not tested in the clinical routine.[69]

Direct effects of antibiotics on intestinal function

Antibiotic agents themselves can directly modulate gut function by interaction with intestinal receptors. Erythromycin, to give an example, binds to motilin-receptors and thus induces contractions in the stomach and duodenum, thereby causing preterm stomach emptying resulting in diarrhoea. A similar mechanism has been suggested for clavulanate acid.[69]

After administration of neomycin, inflammation of the intestinal mucosa leads to symptoms of malabsorbtion and steatorrhoea.[69]

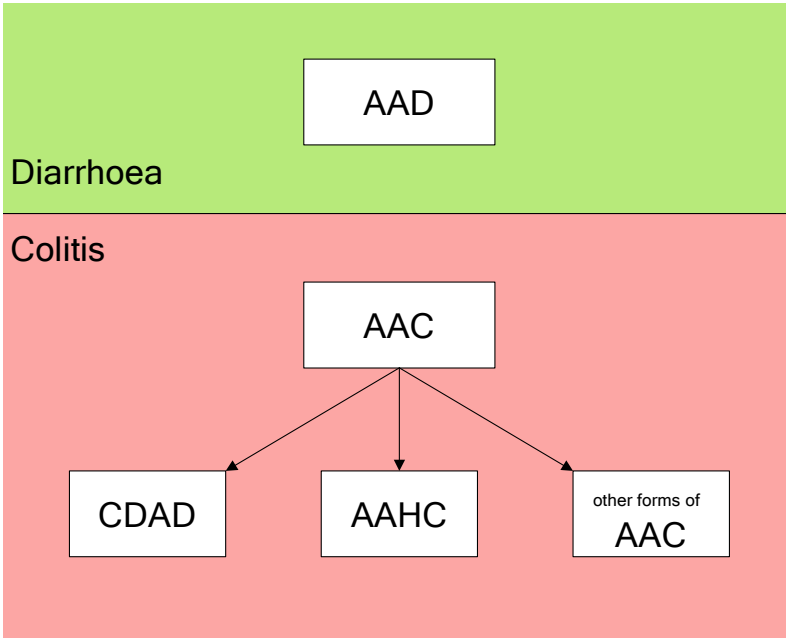
AAD and opportunistic pathogens

The most frequent pathogen causing AAD is *Clostridium difficile*. About 10-20% of cases are caused by this pathogen.[40]Other, less frequently diagnosed bacteria causing AAD are *Clostridium perfringens, Salmonella spp. Staphylococcus aureus and Klebsiella oxytoca* which was recently being held responsible for AAHC.[4 41 42] According to latest studies *Candida spp.* can be regarded as not causative for AAD.[48 69] (also see Figure 2)

Clostridium difficile

The bacteria, because of difficulties to culture historically called *Bacillus difficilis*, is a gram-positive, anaerobe, spore-forming rod equipped with fimbriae and flagellae. It is present in the animate as well as in the unenlivened, people's environment. It is not only found in soil and water but also in the intestinal tracts of animals and humans.[75] *C. difficile* can cause pseudomembraneous enterocolitis (PMC) a severe form of AAC.[2 8 59]

Figure 1: Antibiotic-associated diarrhoea



Antibiotic-associated diarrhoea can be understood as an umbrella term covering other sub-entities of diarrhoea associated with antibiotic treatment.

Infection with *C. difficile* is of increasing concern for the therapist. Strains are becoming more virulent, decreasingly respond to antibiotic treatment and are thus implying an existential threat to the affected.[23 59 74 75] Mortality due to symptomatic infection is stated to be of 1-2.5% and can rise to 20% if infection is caused by the

hyper-virulent *Ribotype O27*.[2 74 75]Age and comorbidity turned out to be crucial determinants of mortality due to CDAD.[2]

Klebsiella spp.

Klebsiella spp. are to be found in the natural environment as well as on mucosal surfaces of mammals including humans. They have been isolated from surface water, sewage, soil and from plants.[66 77] According to their biochemical reaction the genus is defined as being a gram-negative, usually encapsulated, rod-shaped and nonmotile bacteria.

It belongs to the family of *Enterobacteriaceae* which are positive in the Voges-Proskauer test and produce lysine decarboxylase but not ornithine decarboxy-lase.[38 66]

Klebsiella oxytoca and AAHC

First described by Toffler et al. antibiotic-associated-hemorrhagic-colitis (AAHC) is a distinct form of antibiotic-associated-colitis (AAC).[40 84] In contrast to other forms of colitis, abnormalities are mainly located in the right or transverse colon and were observed to be segmental. The condition predominantly affects young and otherwise healthy outpatients having received penicillin derivatives and in some instances quinolones or cephalosporins.

Symptoms such as abdominal cramps and bloody diarrhoea usually commence few days after treatment with antibiotics. Högenauer et al. have recently proven by fulfilling Koch's postulates, *K. oxytoca* to be causally responsible for AAHC. The research group also reported that 10 of 12 patients suffering AAHC were tested positive for K. oxytoca.[40 41]

Differing with *C. difficile* associated colitis the disease is known to resolve spontaneously after cessation of antibiotics.[40 41]

Other bacteria causing antibiotic-associated diarrhoea:

Clostridium perfringens

The pathogen is well known for its ability to cause bacterial food poisoning resulting in abdominal cramps and diarrhoea.[21] [49] Association with antibiotic treatment was not regarded to be crucial.[5] [16] However, a recent study conducted by Asha et al found evidence for *C. perfringens* in 15% of AAD cases.[4]

A variety of human diseases are caused by *Clostridial* species. Diseases are related to potent extracellular toxins.[49]

C. perfringens is a gram-positive, obligate anaerobe, non-motile, encapsulated, spore forming rod. It is a frequent cause of bacterial food poisoning.[38] Type C strains are responsible for necrotising enteritis associated with a very high mortality rate.[49]

Staphylococcus aureus

Staph. aureus plays a major role for infectious disease of the skin. Ranging from relatively mild affections to severe, life threatening disease such as the toxic shock syndrome.[38]

In context with gastrointestinal diseases *Staph. aureus* is not a major bacterium to be mentioned. It is a common cause of bacterial food poisoning though, but association with AAD is rather low. [5] [5] [33]

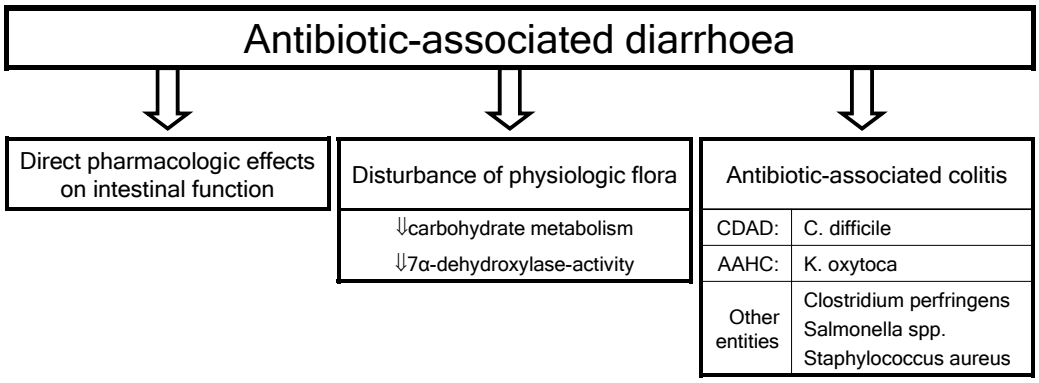
On the other hand 60 cases of *Staph. aureus* AAD could recently be identified by a 2- year prospective study conducted in France.[4] It has been suggested to pay a greater attention on this pathogens in context with AAD.[11] However an animal model for *Staph. aureus* AAD is still missing and evidence for its causative role in AAD therefore remains vague.[22]

Salmonella

Salmonellae are one of the well known food-borne pathogens causing human diseases. As early as 1880 the bacterium associated with Typhus abdominalis has been described by Robert Koch.[33] [33] They cause a wide spectrum of human diseases. *S. enteritidis* strains most common cause a self-limiting *salmonellosis* presenting with watery diarrhoea, fever and abdominal cramps.[49]

Association with antibiotics and gastroenteritis has been reported. According to a study conducted in 1984, 12 out of 18 patients infected with multiresistant *Salmonella newport* have received penicillin derivatives before the onset of diarrhoea.[42]

Figure 2: Suggested pathophysiology of AAD



AAD can either be caused by direct pharmacologic effects of an antibiotic, disturbances of the physiologic microflora or by an infection of pathogenic bacteria. Data from references:[41 74]

Figure 2 summarises the hypotheses on pathophysiological mechanisms leading to AAD mentioned earlier.

Materials and Methods

At the Medical University of Graz twenty patients were diagnosed with AAHC in the period from 1996 to 2008. The data used for the study were retrospectively collected from the case histories.

To confirm the diagnosis, made on the basis of clinical history patients underwent diagnostic colonoscopy (between march 2001 and April 2008). Stools were examined and tested negative for *C. difficile*, *Campylobacter, Salmonella, Yersinia, Shigella, and Escherichia coli O157*. Tests for *C. difficile* were either performed with a stool culture or an ELISA for toxin A or toxin A+B in stool. Presence of all other bacteria mentioned was excluded with a negative stool culture only.
Consistent with typical findings on endoscopy (segmental haemorrhagic colitis) and the absence of *C. difficile* infection, the disease was classified as antibiotic-associated haemorrhagic colitis (AAHC).[41]

Demographic charactristics

The case history was examined closely for demographic data such as reason for antibiotic therapy, age and sex of patients as well as the period of time to the development of first AAHC related symptoms. Special interest was placed on the medication patients had taken before the onset of first AAHC associated complaints. The antibiotic substance prescribed was identified in each case. Whether NSAIDs were taken concomitantly could retrospectively not always be evaluated.

Clinical charactristics

Looking through the case histories particular emphasis was placed on symptoms such as bloody diarrhoea and abdominal pain. Maximal CRP and Leukocyte level was also noted and statistically evaluated.

Endoscopic charactristics

Since the diagnosis of AAHC was made on the basis of clinical history and findings on endoscopy, results from this examination were a decisive factor if patients were included in the study or not.

Microbiologic analysis

Stool samples from patients who received a diagnosis of AAHC were cultured for *K. oxytoca* using MacConkey agar plates. The bacterium was identified with the use of the API 20E test (bioMérieux) and an Indol reaction (bioMérieux). To confirm the classification as *Klebsiella oxytoca* PCR was performed by Weberhofer P.

Evaluation of cytotoxic activity

To substantiate the hypothesis that pathogenic *K. oxytoca* produce a cytotoxin, ultimately responsible for the disease, a MTT assay was conducted.

The MTT assay is a standard colorimetric assay, which can be used to quantify viable cells in a cell culture. Living cells reduce yellow MTT, a tetrazole (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) in their mitochondria to purple formazan. Reduction of MTT does only take place in living cells and is mediated by mitochondrial enzymes. By measuring the absorbance of the solution with a spectrophotometer, a conclusion to the amount of converted MTT can be drawn and thus to viable cells. Hence, suspected cytotoxic effects of a potential toxic agent can be verified if compared to untreated controls.[60]

K. oxytoca culture supernatans

In order to perform the assay bacterial supernatants had to be derived from *K. oxytoca* isolates obtained from patients with antibiotic-associated hemorrhagic colitis.

Tryptic soy broth (TSB) was inoculated with a single bacterial colony and shaken with a frequency of 150 rpm while being incubated at 37°C for twenty hours. Subsequently, cultures were centrifuged at 20.000 × g at 4°C for ten minutes. The

K. oxytoca culture supernatant was filtered trough a membrane filter with a pore diameter of 0.2 µm (Iwaki) and thus extracted. Supernatants were portioned and stored at a temperature of -20°C.

MTT assay

10 *K. oxytoca* supernatants were tested for cytotoxic activity using the established cell-culture assay. For the assay we used eukaryotic HEp-2 cells, which were exposed to the bacterial supernatants assumed to contain bacterial toxins. It was postulated that HEp-2 cells would be altered if toxins were present. Cell death was expected to be reflected by cell rounding, which could be observed under a microscope (see Figure 5) and quantified with the MTT assay as described below. The *K. oxytoca* strain MH 34-1 served as positive control. As negative control we used *K. oxytoca* DSM 5175 (ATCC 13182). The bacterial supernatants derived from patients were kindly provided by Prof. Dr. Högenauer. All isolates classified as *K. oxytoca* were positive, using both the API 20E test and PCR.

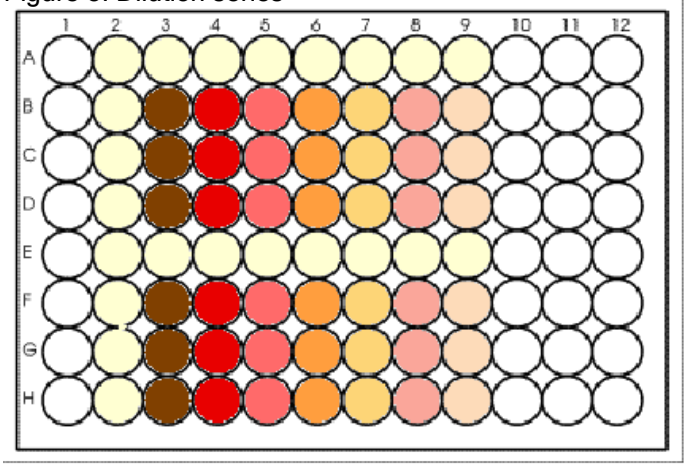
For the MTT cell culture assay HEp-2 cells (ATCC CCL-23) were grown in MEM + 10 % FCS. The trypsin treated eukaryotic cells were seeded into 96 well plates in a concentration of 1.5 x 10^4 cells per well in an end-volume of 100 µl per well. Directly after seeding of the HEp-2 cells the prepared dilutions of the bacterial supernatants were added and HEp-2 cells were incubated in a 5% carbon dioxide atmosphere for 48 h at 37°C. The toxin dilutions were made with PBS (1x) as described below.

Subsequently the culture plate was examined for cell rounding under a microscope. As described previously, cell rounding was considered to reflect cell death and thus cytotoxic activity of the supernatant.

Culture medium was removed and wells were washed with 200µl of PBS. MTT stock solution was added to the wells (200 µl PBS + 25 µl MTT) and incubated at 37°C for one hour. Again the medium was removed and 200 µl of a lysis reagent added. Amount of converted MTT solution was quantified using a spectrophotometer measuring at 595 nm. As described above, quantity of produced purple formazan was assumed to correlate with the amount of viable cells left.

Assay for cytotoxic activity, dilution series

Figure 3: Dilution series



Supernatant was added in wells: B-D, F-H 3-9.
Wells B-D, F-H 2, served as control
Figure kindly provided by Mag. Martina Joainig

As described above 100 µl of cell culture medium, containing 1,5× 10^4 HEp-2 cells were pipetted into seven wells of a 96-well tissue culture plate (see Figure 3). The first well served as control and was not inoculated with supernatant but contained 150 µl of PBS. To the second and third well, 50 µl of supernatant/PBS with a dilution of 1:3 and 1:6 respectively was added. Ending with a dilution of 1:96 at the seventh well. In order to receive reliable results, dilution series of each *K. oxytoca* strain was performed in triplicates, using one culture plate. (see Figure 3) To prevent desiccation of the cells while being incubated at 37°C for 48 hours, the surrounding wells were filled with PBS and the culture plate was capped.

Interpretation of spectrophotometry

Table 2: % of surviving cells

$$\% \text{ of SC} = \frac{MV\ Ab\ SEC}{MV\ Ab\ Con} \times 100$$

SC: survived cells; Ab: absorbance;
MV: mean value; Con: Controll;
SEC: supernatant exposed cells

The percentage of surviving cells was calculated by dividing the average value of absorbance of the supernatant-exposed cells, by the average value of absorbance of the control, multiplied by 100 (see Table 2).

Table 3: Materials

Cell culture medium:	30g / L	trypton soya broth (Sigma) MEM + (minimal essential medium) (with Glutamine, without RNA/DNA) (Gibco)
	10 %	FBS fetal bovine serum (PAA)
	100 x	penicillin/streptomycin (PAA)
Buffer and solutions:		Dulbecco's PBS (PAA) (without Mg and Ca)
	100 x	Trypsin/EDTA (PAA)
Lysis solution:	99 %	acetic acid (Sigma)
		isopropyl alcohol (2-propanol) (Sigma)
MTT stock solution:		25 mg/ml MTT (Sigma) dissolved in PBS
Indol		James (R2) (bioMérieux)

On Table 3 there are listed the materials used for the evaluation of the cytotoxin assay and microbiologic characteristics of *K. oxytoca* strains isolated from patients with AAHC.

Results

As described above twenty patients were diagnosed with AAHC at the Medical University of Graz in the period from 1996 to 2008. Following characteristics could be evaluated retrospectively:

Demographic charactristics

The mean age of patients diagnosed with AAHC was 41 years, ranging from 22 to 62 years. Included were 13 females and 7 males. All patients received antibiotic treatment prior to the onset of symptoms characteristic for AAHC. Two patients were treated with antibiotics for *Helicobacter pylori* eradication. All other individuals received chemotherapeutics for non-gastroenterological reasons. Main indications for treatment with antibiotics were ENT related diseases like sinusitis or tonsillitis.

The mean period of time to the onset of first AAHC symptoms was 6 days, ranging

Table 4. Demographic characteristics of 20 patients with AAHC

Patient	Age	Sex	Antibiotic substance triggering AAHC	Days to symptoms	Indication for antibiotic therapy	Concomitant intake of NSAIDs
B. A.	62	M	Flucloxacillin	7	Furuncle	unknown
B. E.	50	M	Amoxicillin/Clavulanate	7	Bronchitis	No
B. T.	35	M	Amoxicillin/Clavulanate	10	Angina	Yes
E. B	37	F	Amoxicillin/Clavulanate	3	Tonsillitis	Yes
F.W.	50	M	Amoxicillin	3	Sinusitis	unknown
G. O.	22	F	Amoxicillin/Clavulanate	7	Tonsillitis	unknown
G.V.	39	M	Amoxicillin/Clavulanate	7	Sinusitis	Yes
H. R.	34	F	Amoxicillin/Clavulanate	5	Abscession	Yes
H.G.	47	F	Amoxicillin/Clavulanate	7	UT-infection	Yes
H.S.	26	F	Phenoxymethylpenicillin	7	Tonsillitis	Yes
K. E.	36	F	Amoxicillin/Clavulanate	4	Sinusitis	Yes
K. P.	28	F	Amoxicillin/Clavulanate	4	Tonsillitis	unknown
L. M.	42	F	Amoxicillin/Clavulanate	4	Sinusitis	Yes
M. B.	44	F	Amoxicillin/Clavulanate	21	CVC Infection	Yes
P. R.	62	M	Amoxicillin+Clarithromycin	4	HP-eradication	No
R. A.	35	F	Amoxicillin/Clavulanate	4	Maxilla surg.	Yes
R. C.	54	F	Amoxicillin + Metronidazole	4	HP-eradication	Yes
Rt. C.	37	M	Amoxicillin/Clavulanate	7	Sinusitis	unknown
S. E.	44	F	Amoxicillin/Clavulanate	2	Pharingitis	Yes
T. S.	32	F	Ciprofloxacin	10	Sinusitis	Yes

from 2 to 21 days. With one exception all patients received penicillin derivatives as antibiotic substance. More than two third of patients (14 individuals) received amoxicillin/clavulanate. One patient was treated with ciprofloxacin. All other patients (five) received one of the following antibiotics: amoxicillin, phenoxymethylpenicillin, flucloxacillin, amoxicillin plus metronidazole, amoxicillin plus clarithromycin,.

Questioning the concomitant intake of NSAIDs no information could be obtained from five patients. 13 individuals took NSAIDs concurrently with their chemotherapeutic treatment. Only two individuals could explicitly negate such. (see Table 4 for demographic characteristic of 20 patients with AAHC)

Clinical charactristics

Bloody diarrhoea was a major symptom seen in every patient with AAHC. Abdominal pain also was a dominant symptom noted in 16 cases. Only three patients did not experience such pain. No information could be gained in one case. (see Table 5)

Table 5. Clinical characteristics of 20 patients with AAHC

Patient	Bloody diarrhoea	Abdominal pain	CRP	Leukocyte count
B. A.	yes	yes	16	8900
B. E.	yes	yes	8	15400
B. T.	yes	no	55	17500
E. B	yes	yes	16	17600
F.W.	yes	yes	18	16200
G. O.	yes	yes	42	15500
G.V.	yes	yes	29	17200
H. R.	yes	yes	108	6200
H.G.	yes	yes	192	12500
H.S.	yes	yes	-----	21700
K. E.	yes	no	10	11700
K. P.	yes	yes	76	17250
L. M.	yes	yes	34	12740
M. B.	yes	yes	17	27000
P. R.	yes	no	54	15600
R. A.	yes	yes	410	10100
R. C.	yes	yes	39	11700
Rt. C.	yes	yes	18	20230
S. E.	yes	unknown	80	16160
T. S.	yes	yes	16	11900

Ranging from 8 mg/l to 410 mg/l, the median level of maximal CRP was 34 mg/l. The reference value was defined as <9 mg/l. No CRP was measured in one case. The maximal leukocyte levels ranged from 8900 mm^3 to 27000 mm^3 with a mean value of 15325 mm^3. The reference value was <11300 mm^3.

Endoscopic and histologic characteristics

Coherent with clinical characteristics of AAHC, haemorrhage was a consistent finding in endoscopy seen in all 20 cases. Oedema also was observed in 100% of examinations. Lesions were found to be segmental in the majority of cases. Only two patients did not show segmental affections of the colon. But these patients suffered pancolitis. A good third (7 cases) of histologic samples taken showed mucosal ulcerations. (see Table 6)

Table 6: Endoscopic and histologic characteristics of 20 patients with AAHC

Patient	Segments of Colon affected	Segmental	Haemorrhage	Oedema	Ulcera
B. A.	Desc, Transv*	yes	yes	yes	no
B. E.	Rect, Sigma, Transv	yes	yes	yes	no
B. T.	Sigma, Transv, Asc, Coecum	yes	yes	yes	no
E. B	Desc, Transv, Asc, Coecum	yes	yes	yes	no
F.W.	Sigma, Desc, Transv	yes	yes	yes	no
G. O.	Sigma, Transv	yes	yes	yes	no
G.V.	Sigma, Desc, Transv, Asc, Coecum	yes	yes	yes	no
H. R.	Sigma, Desc, Transv	yes	yes	yes	no
H.G.	Sigma, Desc, Transv, Asc, Coecum	yes	yes	yes	no
H.S.	Sigma, Desc, Transv*	yes	yes	yes	yes
K. E.	Asc, Coecum	yes	yes	yes	yes
K. P.	Sigma, Asc	yes	yes	yes	yes
L. M.	Asc, Coecum	yes	yes	yes	yes
M. B.	Pancolitis	no	yes	yes	yes
P. R.	Asc, Coecum	yes	yes	yes	no
R. A.	Transv, Asc, Coecum	yes	yes	yes	no
R. C.	Desc, Asc, Coecum	yes	yes	yes	yes
Rt. C.	Desc, Transv, Asc	yes	yes	yes	no
S. E.	Pancolitis	no	yes	yes	yes
T. S.	Transv, Asc,	yes	yes	yes	no

Rect: Rectum, Sigma: Sigmoid colon, Desc: Descending colon, Transv: Transverse colon, Asc : Ascending colon, *In two patients endoscopy was not performed until the coecum.

Microbiologic analysis

All 20 patients were tested negative for *C. difficile* using either a stool culture or an ELISA-test for toxin A or A&B.? Stools were also examined and tested negative for *Campylobacter, Salmonella*, *Yersinia, Shigella*, and *Escherichia coli O157.*

As described above endoscopy also was performed in all cases and diagnosis of AAHC thereby confirmed. Since some patients were first treated at hospitals other

than the Medical University of Graz or were either diagnosed before 2001, culture and microbiologic analysis for *K. oxytoca* was only performed in 13 patients.

9 isolates were classified as *K. oxytoca*, using both the API 20E test and PCR. Additionally the isolates were tested using an Indol reaction.

1 isolate was classified as *Klebsiella terrigena* and another as *E. coli.* These 2 isolates therefore were excluded.

The PCR was positive for *K. oxytoca* in 9 patients. Indol reaction turned out negative in two cases. In one of these two the API 20E test also was negative. PCR was negative in only one case. (see Table 7)

Table 7: Stool culture isolates from patients with AAHC

Patient	Diagnosis*	Synonym	Isolate number	API	PCR	Indol	MTT assay	C.diff.	Stool culture other pathogens
E.B.	AAHC	AHC-6	# 34	Ko	Ko	+	1/24**	neg	neg
G.O.	AAHC	AHC-8	# 76	Ko	Ko	-	-	neg	neg
G.V.	AAHC		# 118	Ko	Ko	+	1/3**	neg	neg
K.P.	AAHC	AHC-2	# 5	Ko	Ko	+	-	neg	neg
M.B.	AAHC		# 116	Ko	Ko	+	-	neg	neg
P.R.	AAHC	AHC-7	# 56	Ko	Ko	+	1/48**	neg	neg
R.A.	AAHC		# 125	Ko	neg	+	1/24**	neg	neg
R.C.	AAHC	AHC-4	# 17	Ko	Ko	+	-	neg	neg
Rt.C.	AAHC	AHC-1	# 1	Ko	Ko	+	1/3**	neg	neg
S.E.	AAHC				Ko	-	-	neg	neg
T.S.	AAHC		# 180	Ko	Ko	+	1**	neg	neg

*Diagnosis made on the basis of clinical history, endoscopic picture and absence of *C. difficile*; Ko: *K. oxytoca*; **indicating the dilution

Table 8 summarises the relative frequency and localisation of lesions seen in endoscopy in 20 patients with AAHC.

Table 8: Localisation and relative frequency of lesions in 20 patients with AAHC

Localisation	Relative frequency
Rectum	10 %
Sigmoid colon	50 %
Descending colon	50 %
Transverse colon	70 %
*Ascending colon	*65 %
*Coecum	*50 %
*Pancolitis	*10 %

* since in two patients endoscopy was only performed until the transverse colon the relative frequency could be underestimated

MTT assay

For evaluation of zytotoxic activity bacterial supernatants were used for an MTT assay.

The MTT assay was positive in more than 50% of patients (six cases). Two samples each were positive up to a dilution of 1:3 and 1:24 respectively. One of the remaining two was tested positive up to a dilution of 1:48 whereas the last up to a dilution of 1:1. (see Figure 4)

Figure 4: Percent of viable HEp-2 cells exposed to bacterial supernatants

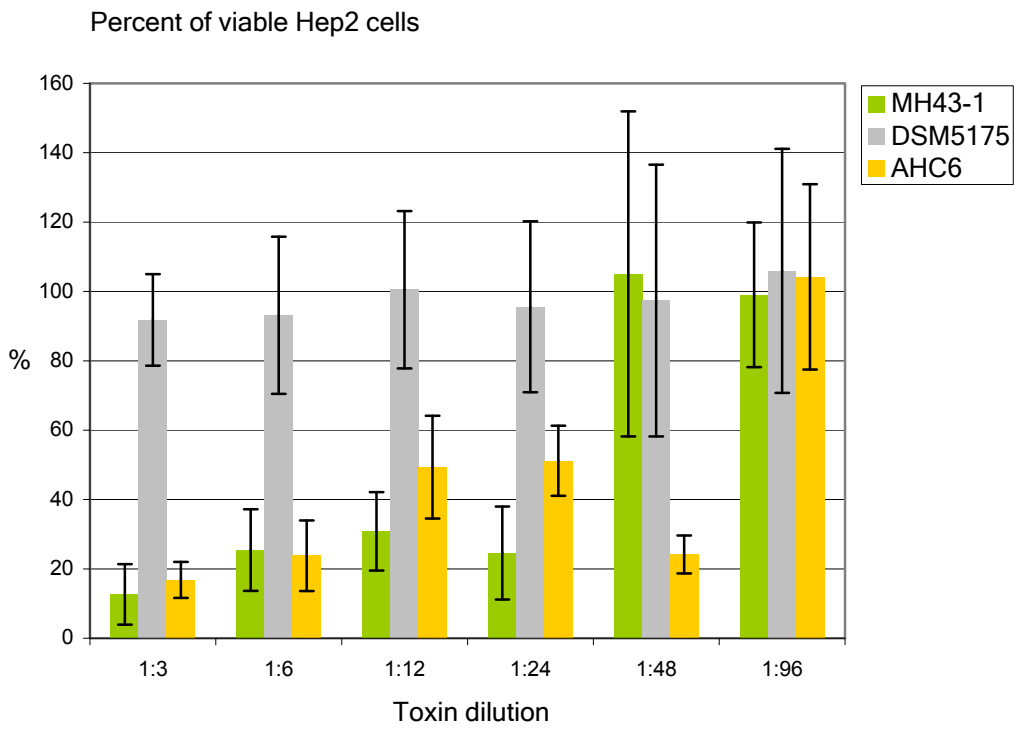
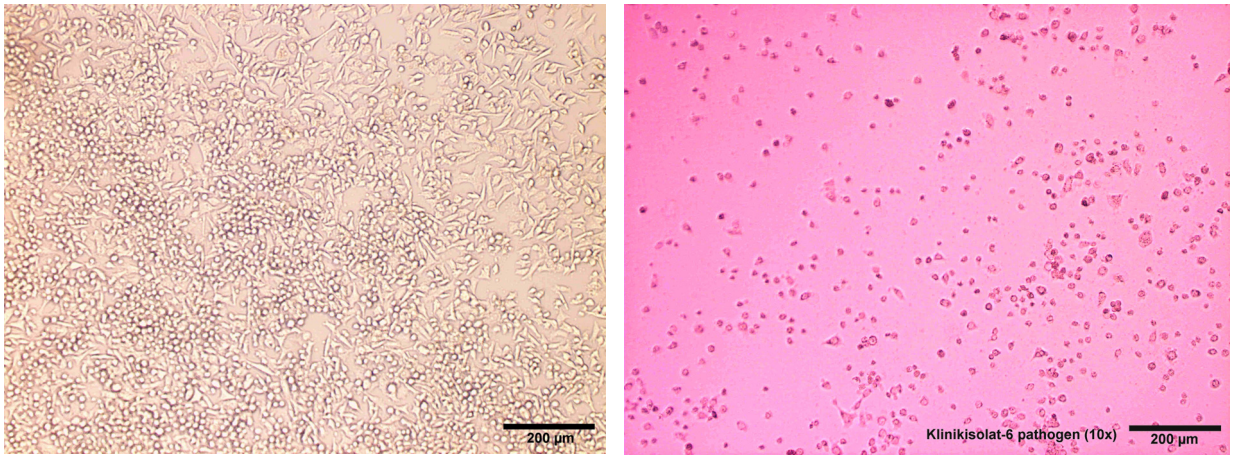


Figure4 is one example of the MTT assays conducted. As described previously HEp-2 cells were exposed to diluted bacterial supernatants. The strain MH43-1 served as positive control whereas DSM5175 was used as negative control. Tested was the strain AHC 6 obtained from a patient with AAHC. Interestingly AHC 6 showed a toxigenic potential at a dilution of 1:48 as high as with a dilution of 1:6

Figure 5: Toxin negative versus toxin positive *K. oxytoca* supernatants on HEp-2 cell cultures.



The HEp-2 cell culture on the left was exposed to toxin negative bacterial supernatants and cells show normal morphology. On the right is to be seen cell rounding of the cells indicating cell death. This culture was exposed to a *K. oxytoca* supernatant derived from an AAHC patient.
Pictures kindly provided by "*K. oxytoca* research group", Medical University of Graz

Discussion

Klebsiella spp.

A discussion on epidemiology, pathophysiology, diagnosis and treatment

Klebsiella spp. infections mostly affect hospitalized, immuno-compromised patients already suffering from severe underlying conditions. The bacteria is an opportunistic nosocomial pathogen and becoming an increasing concern for health care institutions.[52 66] It is being suspected to cause about 8% of all nosocomial bacterial infections.

The most common site of infection is the urinary tract (UTI) for which the pathogens accounts for up to 17% of hospital acquired UTIs. Also the respiratory tract as well as the hepatobiliary system are a well known site of infection.

Paediatric wards are of special concern because *Klebsiella spp.* often are involved in neonatal sepsis.[1 43 66]

In the hospital environment carrier rates of *Klebsiella spp.* are considerably higher than in communities. Rates of stool (77%), hands (42%) and pharynx (19%) emerged to differ significantly from communal analyses where stool samples only ranged between 5%-38% and bacterial counts for the pharynx (1-6%) and hands (only transient colonisation) were much lower.[66]

Högenauer et al. reported to have tested positive for *K. oxytoca* in only 1.6% of stool samples. 385 healthy, asymptomatic individuals were examined in this study. Colonisation rates turned out to increase in direct proportion to the length of hospital stay. Analogical to other bacterial pathogens the use of antibiotics especially of broad-spectrum substances seems to be the underlying cause of these findings. Local antibiotic policy is not only a major determinant of nosocomial *Klebsiella* attack rates but also correlates with occurrence of other multiple resistant strains.

Klebsiellae have a great potential to rapidly spread in hospital settings. As for *C. difficile* the major reservoir for *Klebsiella* epidemics basically are the gastrointestinal tract of patients and the hands of health care workers.

Outbreaks of multiresistant strains would create a very disagreeable scenario in medical institutions. But *Klebsiella* isolates were already reported to produce

extended spectrum beta lactamases (ESBL) in 16% in England and France. Even 40% were mentioned in particular regions. Such high rates alarmingly limit therapeutic options. But physicians could comfort themselves because ESBL positive strains were thought to be sensible to carbapenems such as imipenem or meropenem. Disconcertingly the unhoped-for case did occur. An imipenem resistant *Klebsiella pneumoniae* has emerged, meaning a serious impact for susceptible individuals.

Risk factors for the acquisition of ESBL positive pathogens again are length of hospital stay, poor functional status, having to undergo invasive procedures and ICU stay.[66 76]

Not different to *C. difficile* preventive measures strict adherence to basic hygiene standards such as disinfection of medical equipment, good hand washing practices as well as restrictive use of antibiotics and surveillance is essential to impede further spreading.[15 44 66]

Klebsiella oxytocy and AAHC

Because pathophysiology was unclear until recently many synonymic terms have been used in the past to describe AAHC. Such were: segmental antibiotic-associated hemorrhagic colitis, penicillin-associated hemorrhagic colitis or right-sided antibiotic-associated hemorrhagic colitis. The words used to describe the disease reflect well its endoscopic picture and the correlation with antibiotics.

As already mentioned previously Toffler et al. first described this form of colitis in the year 1978.[40 84]

Although the disease has also been observed in many European countries, Australia and the USA, most reports came from France and Asian countries in particular Japan. It has therefore been suggested that Asian patients were susceptible to acquire AAHC. But there have not been any systematic investigations substantiating this hypothesis. It seems more probable that such impression is due to the fact that in the past Asian research groups have concentrated more on that issue.[40]

Diagnosis of AAHC

Triggers, clinical features and diagnostic tools

Triggers

As the synonym “penicillin-associated hemorrhagic colitis” suggests, AAHC is mostly observed after therapy with penicillin-antibiotics. But cephalosporins have also been observed to be a predisposing factor for the disease.[40 46] In 75% of 20 patients diagnosed with AAHC at the Department of Internal Medicine at the Medical University of Graz, Austria amoxicillin/clavulanate was the triggering antibiotic causing the disease. On average, patients were taking antibiotics for one week prior to onset of AAHC. Almost two-third of the patients were additionally taking nonsteroidal anti-inflammatory drugs.

The stereotypical AAHC patient is a young, otherwise healthy outpatient having received antibiotic treatment.[40]

Symptoms and signs

Leading symptoms of AAHC are: sudden onset of bloody diarrhoea during antibiotic therapy and severe abdominal cramps. Symptoms often are severe and may require hospitalisation.

Elevation of CRP serum levels and mild to moderate leukocytosis was observed in laboratory exams. A few patients even showed very high levels of CRP and leukocytes.[40]

Endoscopic features

Endoscopy is the key instrument to diagnose AAHC. In order not to miss many cases complete colonoscopy to the coecum is necessary since the right colon is mainly affected. But alterations may also be seen in the left colon.

Figure 6: Colonoscopic image of AAHC

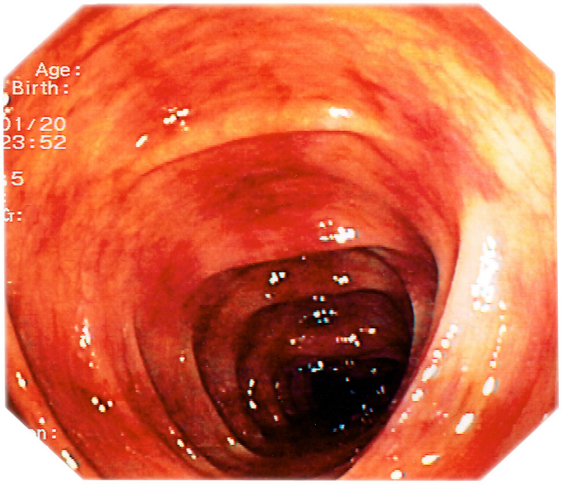


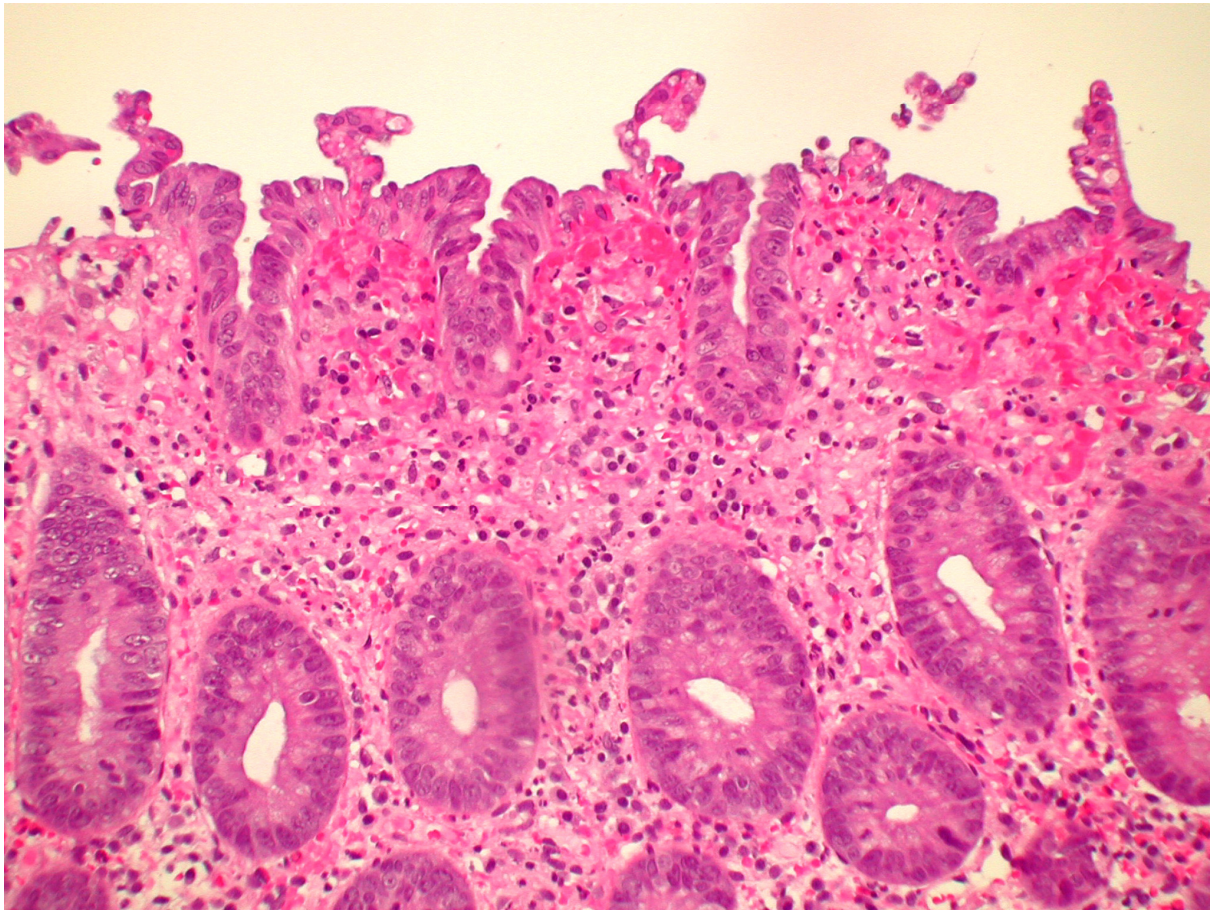
Figure 6 shows oedema an haemorrhage of the colon
Image kindly provided by Prof. Högenauer C.

Although pancolitis may be present in some cases, colitis with mucosal haemorrhage and oedema typically is segmental.[40 45] (see Figure 6)

Histologic features

HE stains of colonic biopsies can be helpful in the diagnosis of AAHC and the differentiation between CDAD. The histological picture may be similar to forms of ischemic colitis.

Figure 7: Histologic specimens of the mucosa of a patient with AAHC



The histologic picture shows the typical epithelial alterations: anisonucleosis, loss of goblet cells, increased rate of mitosis and apoptosis. Also to be seen is mucosal haemorrhage and inflammation. The picture was kindly provided by Prof. Langner C.

Histology of the affected segments shows alterations of the epithelium. An increased rate of apoptosis and meiosis, anisonucleosis and loss of goblet cells can typically be seen. The histological correlate of mucosal haemorrhage is also present patchy distribution.[40 45] (see Figure 7) Mild to moderate inflammation of mucosa with neutrophils invading may as well be present.

It is important to note that histological findings are similar to those found in toxin induced forms of colitis, namely enterohemorraghic *E. coli* or *Shigella spp*. Crypt abscesses as commonly found in infectious colitis due to invasive bacteria are not seen.[40]

Ultrasound and radiographic features

High-resolution abdominal ultrasound may detect asymmetric thickening of the colonic wall and loss of colonic wall layers. Except for older studies using Barium enemas experiences with other radiographic instruments such as CT scans are limited yet.[40]

Microbiological examination

It is most crucial to perform microbiological tests in addition to the endoscopic examination. MacConkey agar plates are commonly used to culture *Klebsiella spp.* of stool samples or intestinal biopsies. Biochemical tests as the API 20E test (bioMérieux) are used for identifying colonies. Since bacterial counts of *K. oxytoca* in stool are very high during the acute phase of illness it is sufficient to perform routine cultures on MacConkey agar plates. Enrichment of samples with ampicillin is not regarded to be necessary. Results are available after 48-72 hours. Microbiological testing alone can not differentiate between a

Figure 8: *K. oxytoca* on a culture plate

Image kindly provided by Prof. Högenauer C.

mere colonisation with *K. oxytoca* and AAHC. Results have to be interpreted in the context of all other findings.[40]

Cytotoxin test

For research purposes cytotoxin production of *K. oxytoca* strains can be assessed by the use of special methods. These tests can not be performed in routine laboratories because they are too time consuming and labour intensive.
(see: “Material and methods”)
Unfortunately routine “direct out of stool tests” as for *C. difficile* are yet not available.[40] If stool samples are negative for *C. difficile*, AAHC should be suspected and stool should be cultured for *K. oxytoca*.[41]

Experimental evaluations of pathophysiological mechanisms of AAHC

Antibiotic-associated hemorrhagic colitis was supposed to be caused only by pathogenic strains of *K. oxytoca*. Colitis was suspected to be mediated by cytotoxines.[36] [41] These hypotheses had to be validated by means of different experimental approaches.

Animal experiment for AAHC

To prove the causal link between AAHC and *K. oxytoca*, Högenauer et al. established an in vivo animal model for AAHC by the use of rats. The experimental group received amoxicillin/clavulanate to suppress the physiologic bowel flora of the animals. Concomitant application of *K. oxytoca* led to colonisation of the organism in the colonic lumen. Histological examination of the animals' intestines revealed that right-sided hemorrhagic colitis was triggered. Features resembled those of human AAHC. None of the control groups got colonised or developed signs of hemorrhagic colitis.[40]

Högenauer et al.: *"Thus, Koch's postulates for infectious diseases were met for K. oxytoca and AAHC. An organism (K. oxytoca) was identified in patients with a clinically well described disease (AAHC). The organism was cultured and induced a similar right-sided colitis in experimental animals. From the diseased colon of the animals, K. oxytoca could again be cultured."* [40]

Assay for cytotoxic activity

From the 1990s different research groups reported that *K. oxytoca* were capable of producing cytotoxic substances.[40]

Recently a report from south China has held *K. oxytoca* responsible for mass mortality of postlarval abalone (Haliotis diversicolor supertexta). This is regarded to be the first report of *K. oxytoca* associated to mortality of abalone.[14]

But pathogenic potential of *K. oxytoca* has not only be linked to sea shells:

Two Japanese groups observed *K. oxytoca* strains from AAHC patients to cause cell death in HEp-2 cells, CHO-K1 cells and HeLa cells.[36] [40] [57] But none of the

control strains showed any toxic effects. Also Beaugerie et al. reported similar findings.[9] Interestingly although is the fact that 47% of strains isolated from asymptomatic carriers also caused cell death in vitro.[40]

As described above we also found cytotoxic activity using a previously developed cell-culture essay.[9]

Suggested pathophysiology

K. oxytoca strains are temporarily present in the human large intestine. Data from investigations in Japan were reported to amount 10% in healthy individuals. If an individual receives certain antibiotic substances the normal bowel flora may get altered and allows *K. oxytoca* to grow. Since *K. oxytoca* constitutively produce β-lactamases and some also extended-spectrum beta-lactamases (ESBLs) the bacterium will get selected by the use of common antibiotics such as amino-and carboxy-penicillins.[30 40] High numbers of pathogenic strains will produce a large amount of cytotoxins resulting in hemorrhagic colitis. AAHC is known to subside spontaneously after cessation of the triggering antibiotic. This probably is due to a regeneration of the normal bowel flora resulting in reinvigoration of colonisation resistance. Thus *K. oxytoca* and its toxins will decrease and hemorrhagic colitis will subside.[40]

Use of NSAIDs has been observed to aggravate infectious diarrhoea and colitis in inflammatory bowel diseases.[19 20 71 80] Similar effects have also been seen in AAHC. It has therefore been speculated that NSAIDs may enhance diffusion of cytotoxins into the colonic wall via increased intestinal permeability.[40]

Treatment of AAHC

Since big studies on AAHC have not been conducted so far, suggestions can only be drawn from small cohorts and case reports. But reports were quite consistent concerning the treatment of AAHC.[40] The disease is observed to subside spontaneously within a few days after cessation of the triggering antibiotic.[29 34] Although there have been some reports that patients were also successfully treated with metroniazole or quinolones it is not regarded necessary to use antibiotics for AAHC. Any antibiotic has the potential to degrade physiologic

bowel flora and thus colonisation resistance. Antibiotics can only be recommended for the severely ill or immunocompromised.

Another open-end question concerns individuals having experienced AAHC in their anamnesis. It is not clear whether for these patients it is ever safe to receive penicillin antibiotics again. If the clinical situation allows alternative antimicrobials it is recommended to avoid penicillin derivatives.[40]

Clostridium difficile

A nosocomial pathogen of increasing relevance

C. difficile is a gram-positive, anaerobe, spore-forming rod equipped with fimbriae and flagellae.[2 59 75]

Pursuant to stool examinations 25-50% of children younger than two years and 70% of newborns are (predominant asymptomatic) carriers of *C. difficile*. In contrast, only 2% of healthy adults are colonized.[8 33 2 33 74] Prevalence of colonised adults in the hospital setting amounts to 25%. More than half of the isolated strains are toxin producing.[2]

The bacterium is responsible for up to 20% of cases of AAD and virtually all cases of pseudomembraneous colitis.[40 75]

Mortality due to symptomatic infection is stated to be of 1-2.5% and can rise to 20% if infection is caused by the hyper virulent *Ribotype O27*.[2 74 75]

Age, hospitalisation and co-morbidity turned out to be crucial determinants of mortality due to CDAD.[2 7]

Epidemiology, transmission, risk factors

Reservoir and transmission

C. difficile is present in the animate as well as in the un-enlivened, people's environment. It is not only found in soil and water but also in the intestinal tracts of animals and humans.[2 79] As already mentioned, 80% percent of newborns are colonised with the bacterium. The absence of *C. difficile*-toxin binding receptors on the surface of colonists probably gives an explanation for the fact that babies

usually do not develop symptoms. With growing age the prevalence of colonisation decreases but susceptibility to CDAD notably rises.

The main source of transmission remains the CDAD-patient and its environment. Significantly more spores were found in the immediate vicinity of CDAD patients than in only colonised ones.[2 86] An affected individual suffering from severe diarrhoea represents the worst possible case in terms of infection control measures. Patients excrete an amount of 10^{7-9} *C. difficile* organisms per gram of stool. 10-40% of CDAD patients remain colonised after symptoms have subsided.

Exposed individuals mainly inoculate the spores of CDAD patients. The risk to get infected is high. According to animal models two *C. difficile* cells might be sufficient to result in infection. CDAD has also been described in animals such as horses, dogs, cats, birds and others. A zoo-anthroponotic transmission seems probable. It might happen via a direct or indirect faecal-oral transmission i.e. through contaminated foods.[2 28]

The spores of the organism persist up to 40 days in the hospital environment. Spores are inactive, highly resistant forms of the bacterium which usually survive routine hospital disinfection.[23 26] They can be found on hospital floors, bed rails windowsills, toilets and most important, hands of health care workers.[8 33 38 74 75] Under the right circumstances as in weakened human intestines they change to vegetative morbid pathogens[23]. The acquisition rate of *C. difficile* in hospitalised patients is estimated to be 13% upon completion of two weeks and escalates as high as 50% measured after one month.[17] At an average of 3.2 days, patients sharing a room with an infected individual get contaminated, compared to 18.9 days if not residing in the same room.[54] Immunoplot typing methods proved that infection is predominantly spread by the hands of patients and health care workers. 75% of physicians were contaminated after having been in contact with affected patients.[23]

Epidemiology

Not only the incidence of CDAD has risen in the last five years but also severity, risk of recurrence and lethality of the disease have notably increased over this only short period of time.[2 64 67 68] For eight European countries, including Germany, the

average incidence of CDAD per year was estimated to be 110 per 100.000 admissions. In many European hospitals CDAD is the most common nosocomial infection already exceeding diseases due to *methicillin-resistant Staphylococcus aureus (MRSA).* Numbers of lethal cases in England were double as high as to MRSA in year 2003.

In the time between 2000 and 2003, CDAD incidence in the USA amounted to 228 per 100.000 inhabitants older than 65 years of age. For people between 45 and 64 years of age the incidence was 40 per 100.000.

Incidence of community acquired CDAD has also risen. 22 cases per 100.000 inhabitants were recorded in England in the year 2004 compared with 1994`s incidence of only 1/100.000.

With accumulation of CDAD cases financial charges for the health care system become soar. According with estimates made, average cost over-runs for CDAD patients account for 54%. This is due to the intense extra work necessary for CDAD cases.[2]

Risk factors

The human digestive tract is exposed to numerous, potentially pathogenic organisms. The bowel flora, gastric acid, the mucosa and its immune system form an effective barrier against colonisation and infection. Common substances that can possibly alter these defend mechanisms are antibiotics, chemotherapeutics and PPIs. The exposition to an high risk environment such as health care institutions, combined with an impaired colonisation resistance of the individual dramatically augment the probability to develop disease. Treatment with antibiotics is the most common and therefore most relevant risk factor concerning preventive measures. A nosocomial outbreak of *Ribotype 027* associated disease in the year 2006 revealed that fluoroquinolones and cephalosporins were the most dominant risk factors to develop CDAD. It is

Table9 Risk factors to acquire *C. difficile* associated diarrhoea

- advanced age
- antibiotic therapy
- cytostatic therapy
- immunosuppressive therapy
- nasogastric tube
- prolonged hospital stay
- residing in a nursing home
- severe co-morbidities
- stay at intensive care unit
- surgical procedures
- use of antacids
- sharing a room with a *C. difficile* infected person

Information from references:[2 23 75]

suspected that the increasingly used fluoroquinolones will forward *Ribotype 027*-spreading.

Vancomycin a glycopeptide effective against *C. difficile* and its virulent *Ribotype 027* is even suspected to avail infection with *C. difficile*. That probably is because no antibiotic acts against the spores but always affects the physiologic bowel flora and therefore colonisation resistance.

Even though antibiotics may play a key role in CDAD all factors striking the intestinal barrier, namely the epithelium, the immune system and the normal flora influence its resistance against pathogens.[2]

Patients at highest risk to acquire *C. difficile* infection are generally those with an altered immune system irrespective of the aetiology.[75] Individuals older than 65 have a 10-fold higher risk of getting infected compared to others.[74] Considering all stated above it is evident that *C. difficile* implies a serous health threat for the susceptible population. Though, the disease remains comparatively un-noted.

John S. Fordtran:

"…epidemic's don't create an outcry if only grandmothers are dying."[23]

Ribotype 027

The awareness that the bacterium *C. difficile* has to be taken seriously for public health grew immensely after the emergence of a highly virulent strain called *Ribotype 027*. It was first isolated in France in 1988. It is believed that an acquired resistance against fluoroquinolones has enabled the worldwide spreading of the aberrant type. Dramatic outbreaks have been observed in Canada and the USA. Today *Ribotype 027* has also been detected in the EU and Austria. The first case observed in Austria in March 2006 was a 69 year old female who probably had acquired the bacterium in England. Because of bronchitis the British tourist had been treated with antibiotics ante-arrival.[2]

The strain is characterized by a deletion of a gene regulating the toxin production. It shows impaired response to standard antibiotic treatment and is resistant to fluoroquinolones. Severe clinical courses with high rates of recurrence and mortality are associated with it. Mortality can amount to 20%.[2]

CDAD and PPIs

Use of antacids has been linked to increased risk to acquire CDAD.[18 83]

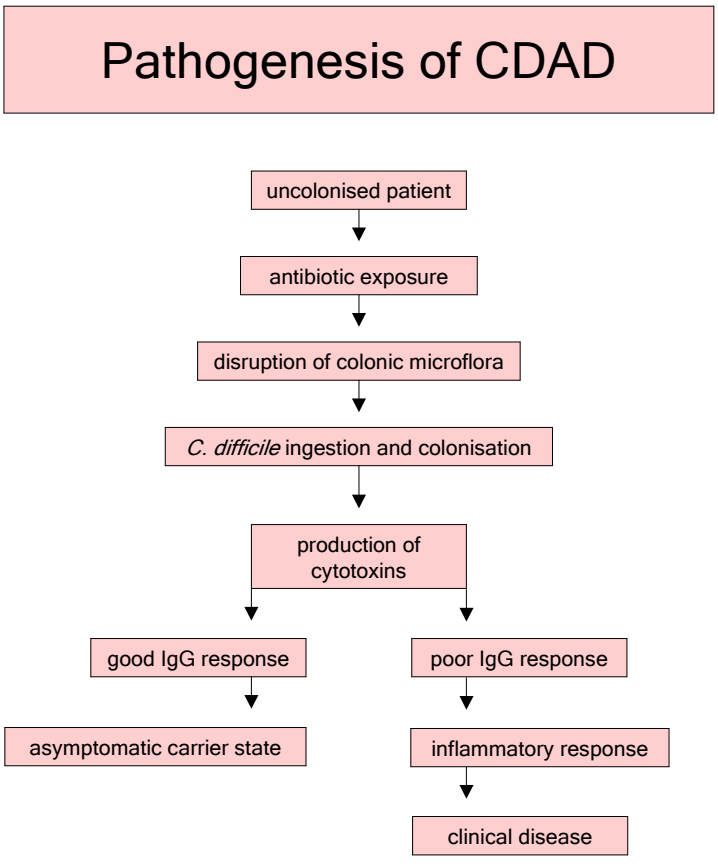
Gastric acid is a natural barrier for bacterial pathogens as it kills many of the vegetative forms. If gastric pH is augmented it can no longer serve its purpose and acid-sensitive pathogens are more likely to survive. Commensals of the colon are probable to ascend and gain a foothold in the upper intestine if the gastric milieu is disturbed. Such hypotheses are substantiated by studies observing that the by-passing of the stomach i.e. via a post pyloric probe is associated with a high relative risk to develop CDAD.[2]

Many patients regularly take PPIs not only because they need it but also because of direct-to–consumer advertising or just because they never stopped to take a drug they once got prescribed. Intensive care patients nearly all are on PPIs because of the fear to induce stress ulcer bleedings. PPIs have actually proven to reduce already low rates of bleeding in intensive care units but according to a meta analysis mortality rates have risen.[23] A case control study recently conducted has found a strong association with community acquired *C. difficile* infection and the use of PPIs.[2]

Suggested pathophysiology of CDAD

Depending on host factors, manifestation of the disease ranges from asymptomatic carriage and mild diarrhoea to severe pseudomembranous colitis. It can develop up to eight weeks after cessation of antibiotic treatment. Colonisation occurs through the ingestion of spores which convert to vegetative forms in the colon after disruption of the microflora by an antibi-

Figure 9: Suggested pathogenesis of CDAD



Information from reference:[75]

otic.[8 23 33 74 75] Constrained by factors of the pathogen and the host, clinical manifestation may develop.[8] As mentioned later [23 33]the disease is caused by at least two toxins (cdTA&B), produced by the pathogenic *C. difficile* strains.[8] If it lacks the gene for toxin production, no symptoms will occur. Non-toxic *C. difficile* do not cause disease. It was suggested that symptoms will only be mild if the host's immunological respond is brisk and effective[23]. However, it is controversially discussed weather antitoxin antibodies really are capable of inhibiting the course from colonisation to symptomatic infection.[2 73]
(see Figure 8)

Clinical symptoms and diagnosis of CDAD

Laboratory tests

Since correct diagnosis is crucial for correct and effective treatment (*E. coli 0157:H7*-associated diarrhoea treated with antibiotics would even worsen the situation) it is helpful to have good laboratory methods.[23]
The most common is an **ELISA** for toxin A and B.[6 55] Results are available within a few hours and have a good specificity. However, sensitivity is only around 70 %.[23 74 75] Since results remain positive for some time, the assay can not be used as an indicator for successful treatment.[75]

A **stool culture** followed by a toxin assay of *C. difficile* colonies probably is the most sensitive method for laboratory diagnosis.[81] The method is highly sensitive and specific but unfortunately difficult to perform and results can take up to 48 hours.[23 74 75]

Clinical symptoms

General symptoms can be quite diversified. Ranging from mild to severe diarrhoea, low-grade to high–grade fever, with or without leukocytosis. There can be abdominal cramps, hypovolemia, shock and hypoalbuminaemia, if albumin

Table 10 Defining characteristics of CDAD and AAHC

	AAHC	CDAD
Common causative antibiotics	Penicillins	Clindamycin Amoxicillin Cephalosporins Quinolones
Typical patients	Outpatient Young age	Inpatient Older age Severe Comorbidities
Clinical characteristics	Bloody diarrhoea, Sudden onset Abdominal cramps	Watery diarrhoea Usually no blood
Endoscopic characteristics	Right colon Segmental colitis Oedema Haemorrhage Ulcers occasionally	Left colon Continuous colitis Pseudomembranes or Unspecific colitis
Causative pathogen Toxins	*K. oxytoca* Unknown toxins	*C. difficile* CdT-A/B
Diagnosis	Endoscopy Stool culture	Test for CdT-A/B Endoscopy Stool culture
First line therapy	Terminate antibiotic Therapy	Terminate causative antibiotics and/or metronidazole vancomycin

Information from references:[40] [40]

oozes into the colonic lumen.[7] [23] [74] Vomiting is rare. Watery diarrhoea with pain in the lower abdominal quarters and a putrefactive smell of stool as well as other general symptoms of infectious gastrointestinal disease have been described to be typical.[2]

Also **stool** samples show a variety of signs. They can be bloody (in 30% occult), contain leukocytes but can also be normal.[23] Major characteristic of CDAD and AAHC are contrasted on Table 10.

Complications and recurrence of CDAD

Complications

Complications involve dehydration, hypotension, fulminant, pseudomembraneous colitis and toxic megacolon, but also perforation or paralytic ileus. If that is the case, there may not be diarrhoea![23] [74]

Since symptoms may occur many weeks after cessation of antibiotic treatment a detailed clinical history with emphasis on antibiotic use is crucial for early diagnosis. Stool abnormalities need to be inquired about. Co-morbidities can lead to suspect *C. difficile* infection. These include a history of fever, a recent surgical procedure, immunosuppressants or contact to infected individuals.[23] [75]

Table 11 Indices of clinically severe CDAD

Conditions	Values
Age	>65 y
Development of CDAD	Nosocomial
Hypotension/shock/SIRS	Yes
Lactate	>2,2 mmol/l
Leukocytosis	>20.000 G/l
MODS	Yes
No response to therapy	Yes
Paralytic ileus	Yes
Severe underlying disease	Yes
Toxic megacolon	Yes
Vasopressor dependence	Yes
Co-therapies	Immunosuppressives, Cytostatic therapy

Information from reference:[2]

Endoscopy and histological examination may be needed for clarification, especially if laboratory testing was negative. Though, it can not be used if fulminant colitis is suspected because of its risk of perforation. The pseudomembranes may not be seen in every case of pseudomembraneous colitis since they can get washed out during pre endoscopic treatment. Histologic biopsies remain reliable though.[69 74] Indices of severe CDAD are listed on table 11.

Recurrence

Recurrence of CDAD is defined as a second episode of diarrhoea or toxic megacolon with *C. difficile* or its toxins present in stool 2 to 8 weeks after the onset of the first episode that primarily had been subsided.

In daily routine it is difficult to distinguish between recurrence of CDAD or new infection with a different strain. If symptoms develop within 8 weeks it shell be classified as a recurrent case. New infection will be assumed after 9 weeks of first onset (independent of the strain detected).

Recurrent cases seem to have become more prevalent over the years. It is unclear whether this is due to the emergence of antibiotic resistant strains like *Ribotype 027* or other factors of virulence independent of resistance to chemotherapeutics. Higher awareness of clinical staff was also suggested to play an important role. On average 5% to 35% of CDAD patients develop a recurrent disease.

It is important to mention that risk of recurrence is independent of prior therapy with antibiotics. Also metronidazole and vancomycin are similarly often associated with recurrent cases.

The risk to develop recurrent CDAD is associated with the following factors: older than 65 years of age, low antibody titres, blockers of gastric acid, stool incontinence, and number of previous recurrences.[2 13]

Low titres of antitoxin antibodies are associated with colonisation of *C. difficile* and recurrence of CDAD. IgG-antibodies against *C. difficile* toxin A have been detected in symptom free carriers. It is believed that antitoxin A-antibodies are capable to prevent the progression to symptomatic disease. It was suggested that low titres of antibodies may be responsible for recurrence. Patients suffering recurrence of CDAD had titres of 0,7 IU/ml whereas individuals experiencing the first episode of CDAD had titres as high as 6 IU/ml. Carriers showed intermediate levels of 3 IU /ml. A case series of PMC patients identified the use of gastric acid blockers as the most frequent risk factor to develop CDAD recurrence.[2]

Treatment of CDAD

General measures, like administration of fluids and electrolytes as well as discontinuation of antibiotics in otherwise healthy individuals can resolve nearly one quarter of cases.[75] All antiperistaltic agents should be avoided because they prolong transit of toxaemic bacterial substances.[23 74 75]

Specific pharmacotherapy is recommended for most cases of CDAD except for mild and self-limiting ones. If the patient depends on antibiotic treatment due to its underlying illness, substances not suspected to cause diarrhoea shall be preferred.[8 33 75]

Antibiotic treatment

Metronidazole vs Vancomycin

First line therapy consists of metronidazole 500 mg tree times daily per os for 10 to 14 days.[2] Intravenous administered metronidazole is also effective since 15% are secreted into the colon (personal communication with Prof. Högenauer C.). Therefore vancomycin orally remains second line therapy because of much greater costs and the risk to induce resistances, especially vancomycin resistant *enterococci*. It will be used for relapses (89% toxin elimination vs. 59%, compared to Metroniazole),[74] if metronidazole failed to be effective or if side effects as

nausea and peripheral neuropathy compel to replace substances. Additional, vancomycin orally is used for pregnant or breastfeeding women[2 23]If so, it should be started with 125 to 500 mg orally 10 to 14 days, four times daily.[23 74 75]
Response rates were reported to be as low as 75% in some series.[74]
A recent study also found significantly higher efficacy for severe *C. difficile*-associated colitis and *Ribotype 027* associated disease compared with metronidazole.[2]

Teicoplanin (Targocid)

The substance shows good initial response rates (96%) and even better relapse rates (7%) compared to metronidazole or vancomycin. According to a Cochrane review teicoplanin was regarded to be the best choice in terms of the patient's clinical condition and prevention of spread of *C. difficile* infection.[61] 400 mg will be given twice daily for 10 to 14 days.[74] However the therapy is very expensive and therefore reserved for selected cases.[2]

Fusidic acid (Fucidine)

Fusidic acid (500mg, three times daily for 10 days) proved to be effective (83%-93% response rate) but showed to have higher relapse rates with up to 27%.[2 74]
Also there is evidence suggesting that selection of resistant strains was frequent with the use of fusidic acid.[62 63]

Rifampicin (Rimactan)

For rifampicin there were no randomised trials conducted. But clinical case reports refer to the effectiveness.[74]

Rifaximin

Also the use of Rifaximin is suggested for recurrent CDAD.[47]

Additional therapies

Probiotics

For prevention of *C. difficile* associated diarrhoea probiotic agents have shown positive effects in some studies.[35 53] But for treatment of CDAD they have yet not been proven to be affective.[65] For the treatment of relapses though, *Saccharomyces boulardii* in combination with metronidazole or vancomycin turned out to be potent.[25 74] A similar effect was also stated for oligofructose.[74]

Adsorbent agents

Data concerning cholestyramine remain contradictory. But they should be avoided for the combination with orally administered vancomycin since its effective concentration will be reduced.[74]

Tolevamer

It is a high molecular weight polymer and an anionic adsorbent that proved to have efficacy as good as oral vancomycin.[2 50]

Immunologic therapies

Patients having had AAD tended to be more susceptible to relapses if they had low levels of IgG against cdTA. Multiple smaller studies could also prove immunoglobulines to be efficacious. But as long as no randomised controlled trials are available such treatment can not generally be recommended. Although not yet sufficiently proven immunoglobulin milk powder, given orally could become an auspicious alternative.

As prophylactic therapy vaccines against cdTA/B shall additionally be mentioned.[74]

Enemas of human stool

Numerous case reports suggest treatment with human stool. The idea of this therapy is to restore the normal bowel flora. Some report excellent response rates and have prevented the patient from colectomy though there is a potential risk of transmitting infectious agents such retroviruses. Stool therapy was mainly used for desperate relapsing cases.[51 74 75]

Surgical intervention

If medical therapy fails and AAD develops to severe, complicated disease with toxic megacolon or perforation surgical treatment is the ultimate option to take. In such cases surgical intervention should be performed quickly.[32] If so, total or subtotal colectomy with ileostomy will be conducted. Surgical intervention is associated with very high mortality, especially if perforation has already occurred. One study found a 100% mortality rate after hemicolectomy.[2 12] Therefore total or subtotal colectomy should be performed. A retrospective analysis could prove a benefit of surgical intervention for the following subgroup of CDAD patients: age >65 years, maximum leukocyte count >20.000 G/l, maximum lactate in serum >2,2 and <5mmol/l.[2]

Response and relapse

Resolution of fever and diarrhoea within two to four days is a good sign for efficacious therapy. But treatment should not be determined before five days despite prior ineffectiveness.[75]

Recurrent infection occurs in nearly one quarter of cases. It is usually not caused by treatment –resistant strains but by germination of persistent spores or reinfection via reingestion.[8 33 75]Treatment of relapsing cases shall be deemed to be unclear. Some options were already discussed above but alternating application of vancomycin pulse and taper was shown to be effective in one controlled study.[74 75]

Prevention of CDAD

Preventive measures to reduce infection include the following: restrictive allocation of antibiotics, especially concerning the combination or use of a broad spectrum substance. In order to limit further spread, infected patients need to be detected early and be isolated from the uninfected. Use of gloves, rigorous, proper disinfection and washing standards, both furniture and hands reduce risk of widespread contamination.[3 8 23 33 38 75]It is important to mention that alcoholic disinfectants and many other common agents are not adequate to kill spores. Hand disinfection without washing is therefore not sufficient!

To assure all measures to be accomplished in daily routine, regular training of hospital staff is necessary.[2]

Identification of CDAD patients is crucial for action taking. Any individual in the hospital setting suffering from diarrhoea should be tested for *C. difficile*.
It remains controversial whether a screening for *C. difficile* colonisation of symptom free patients would have any positive impact on the situation. There is no evidence that antibiotic therapy could entirely eradicate the bacterium.[2] [26]
Furthermore it was also suggested that colonisation could be a protective factor against the onset of CDAD.
To concentrate on the symptomatic patient with diarrhoea remains most important in terms of transmission control.
The European CDAD-guideline recommends implementing a CDAD-surveillance-system in every health care institution.[2]

Isolation of CDAD patients is a very effective measure to minimize risk of transmission to other patients, staff or attendants. Patients should be accommodated in single rooms with their own bathroom and toilet. If several individuals suffer CDAD they may be displaced to the same room. In case of a CDAD outbreak in an institution it might be necessary to isolate entire wards and to allocate staff exclusively responsible for these. Infected patients should not leave wards before 48 hours after symptoms have ceased.
Contamination of the environment via spores can be decreased due to such actions.[2]

Hygiene of hands is an important issue in view of infection control strategies. Johnson et al. have revealed its significance in the year 1990. The use of non-sterile, disposal gloves could reduce CDAD incidence from 7,7 to 1,5 per 1.000 admissions. Gloves need to be worn at each contact with the patient or his/her environment.
As already mentioned, alcoholic disinfectants are ineffective against spores. Studies have proven that disinfection of hands alone could not decrease CDAD incidence. It is also necessary to wash hands for 10 seconds after disinfection. The water will wash away the spores while disinfectants will have killed the

vegetative forms of the bacterium before. It is not recommended to first wash and then disinfect. The vegetative forms in the washing basin could be a source of new spores and transmission.
Also the patient himself/herself is called upon to adherence to proper personal hygiene.

Protective closing is highly recommended to be worn while in contact with CDAD patients, their excrements or proximate environment. Coats or skirts will be sufficient.[2]

Disinfectants for the use of surfaces must be effective against spores. 10% sodium hypochlorite was shown to be effective.[26]To ascertain such sporocidal activity agents must have undergone testing. It is recommended to prove labelling of agents. Accepted standards - to name a few - are: prEN 14347, NF T 72-230 and NF T 72-231. More detailed information on hygiene standards, disinfection of surfaces and medical devices is provided by the Austrian health and nutrition safety agency (see references).[2]
The combination of hygiene measures showed high effectiveness in several studies conducted. A decline from 155 down to 67 CDAD cases per year was described. This means a reduction of 60%.[2] Also Struelens et al. reported a drop of 73% after combined actions had been taken. CDAD cases per 1.000 admissions were reduced from 1,5 to 0,3.[82]
As described, combined measures include testing for CDAD, restrictive use of antibiotics, isolation, surveillance, education of staff, the wearing of protective clothes, intensifying hygiene of hands, surfaces and medical devices.[2]

Bacterial toxins and other factors of bacterial pathogenicity

Knowledge about pathophysiological mechanism of bacterial toxins has grown continuously in the last decades. For instance much is known about major virulence factors of *C. difficile* toxins and immunologic responses of the body itself.[27]

For AAHC on the contrary detailed insight into pathophysiology remains scarce. Alterations of the colonic wall and death of various cell culture lines has been linked to cytotoxic activity of *K. oxytoca* products. Albeit data indicating such, the toxin itself could yet not be identified.[40] In order to characterize the molecular structure of toxigenic substance produced by *K. oxytoca* a lot of efforts will have to be made. With emphasis on *C. difficile* and *Klebsiella spp.* literature on bacterial factors of pathogenicity will be discussed in the following.

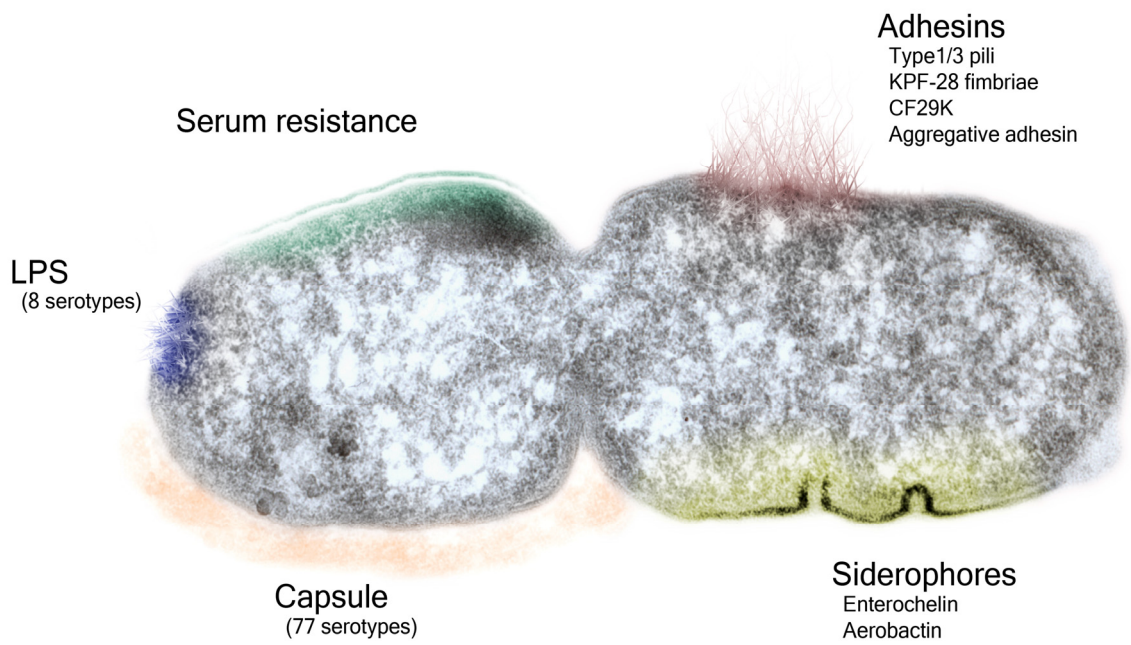
Klebsiella spp.

Capsule

Complex acidic polysaccharides form thick bundles of fibrillous structures covering the bacteria into massive layers and being essential for the virulence of *Klebsiellae.*[37] [66] Virulence is understood as the degree of pathogenicity of the strain. The capsular repeating subunits predominantly consist of negatively charged uronic acids and chains of four to six sugars. They can be classified into 77 serological types. The prominent capsule does not only protect the bacterium from phagocytosis by granulocytes but also prevents killing by bactericidal serum factors. Presumably via inhibition of complement activation, especially C3b. Additionally the capsule is being held responsible to impede the differentiation and functional capacity of macrophages in vitro. Injection of large doses of *Klebsiella* capsular polysaccharide (CPS) may even lead to immunological paralysis. As demonstrated in a mice model, application caused a dose–dependent decrease in the production of specific antibodies.

As already mentioned, capsular types can be differentiated by K antigens into 77 subtypes. Though only few of the 77 subtypes have been systematically studied. The K antigens are associated with virulence. For instance K1 and K2 are regarded as being of especial virulence. This is probably due to a divers content of

Figure 10: Pathogenicity factors of *Klebsiella spp*.



The image was modified after informations from referece:[66] The original picture of the bacteria was kindly provided by Prof. Gorgiewicz G.

mannose in the CPS. Capsular types containing repetitive sequences of mannose-α-2/3-mannose or L-rhamose-α-2/3- L-rhamose (e.g.: K7, K21a) are known to be less virulent. That can be explained by the fact that these repetitive sequences are recognised by surface lectin macrophages mediating opsonin-independent phagocytosis, so called lectinophagocytosis. That is a nonopsonic phagocytosis (e.g.: complement-and antibody-independent) mediated by surface lectins on one cell and surface carbohydrates by the other cell. Mannose- α-2/3-mannose-specific lectins or mannose receptors on macrophages recognise the *Klebsiella* repeating sequences mannose-α-2/3-mannose and L-rhamose-α-2/3- L-rhamose on capsular CPS. Hence they will be killed by getting ingested. *Klebsiella* lacking such sequences can escape phagocytosis because macrophages are unable to recognise the bacteria. Consequently K2, not exposing mannose-α-2/3-mannose is causing disease. For the respiratory tract mannose-α-2/3-mannose also has importance for its clearance. Surfactant protein A enhances phagocytosis of

mannose-α-2/3-mannose positive strains such as K21a by alveolar macrophages. For K2 such enhancement could not be found. The reaction was inhibited by mannan leading to the hypothesis that bacterial binding is mediated by the macrophage mannose receptor.

Attempts to correlate serotypes to specific organs did not provide consistent results. However K2 did again turn out to be the most dominant subtype in human infections. It was isolated from urinary tract infections, pneumonia and bacteraemia. But it is very rarely found in the environment.

Adhesins

To dock on the mucosal host cells, *Enterobacteriaceae* generally use pili or fimbriae. These are nonflagellar, filamentous projections up to 10 µm long with a diameter between 1 and 11 nm. They are located on the bacterial surface and consist of polymeric globular protein subunits. Molecular mass is 15 to 26 kDa.

Type1 and type 3 pili are the predominantly produced sorts of adhesins produced by *Klebsillae*. Pili can be differentiated by their ability to agglutinate erythrocytes and whether the reaction is inhibited by D-mannose or not.

Type 1 pili

Also known as common pili, type 1 pili agglutinate erythrocytes and are mannose sensitive haemagglutinins (MSHA). Being capable of binding to mannose – containing trisaccharides of the host's glycoprotein the adhesion protein is located on the fimbrial shaft and mainly attaches to mucus or epithelial cells. Common site are the urogenital, respiratory and intestinal tract but also the proximal tubus cells are known to be susceptible.

Presumably the sugar structures consist of short oligomannose chains. They are believed to be bound via N-glycosidic linkages to the glycoproteins.

Type 1 pili also adhere to mannosyl-containing glycoproteins in urine and saliva and therefore promote variable bacterial colonisation. Such colonisation in the respiratory tract can again abet pathogenic overgrowth. Especially affected are weakened individuals. Patients undergoing long term mechanical ventilation for example easily acquire pneumonia under these premises.

Common pili are no longer of use for the bacteria after adhesion to the target surface because then it triggers lectinophagocytosis, meaning death for the pathogen. Since the leukocytes have specific mannose-containing receptors on their surface the hydrophilic pili would bind to them. Subsequently phagocytosis would be activated via this trigger. The pathogen therefore switches off the expression of type 1 pili once it reaches host surfaces.

Type 3 pili

These pili are able to adhere to various cells not only of humans but also of plants. They were described to attach to cells of the respiratory tract and to different uroepithelial cell types. In contrast to other fimbriae they agglutinate only erythrocytes treated with tannin. They are mannose-resistant and show a large antigenic diversity.
But so far the impact on pathogenicity is not known.

Other adhesins

KPF-28, a recently detected fimbria probably is a colonisation factor of the gut. It is predominantly found on ESBL positive *Klebsiella pneumoniae* strains producing CAZ-5/SHV-4.
CF29K, a non-fimbrial adhesin also produced by *K. pneumoniae* is known to stick to human intestinal cell lines. Interestingly it seems to be identical to CS31-A adhesive proteins of *E. coli* strains associated with diarrhoea. The R-plasmid-encoded CF29K adhesin probably is a product of transfer of CS31A from *E. coli* to *K. pneumoniae.*

Lipopolysaccharides

Invading pathogens are defeated by the bactericidal activity of the serum once they get in touch with it. This is primarily mediated by complement proteins. Such proteins are able to produce a transmembraneous pore in the outer membrane of gram-negative bacteria and thus causing a Na^+ influx leading to osmotic lysis of the bacteria. The transmembraneous membrane attack complex consisting of terminal complement proteins (C5b-C9) and is the final step of an elaborate cascade-like activation. It can be activated by two different mechanisms. The

classic complement pathway requires specific antibodies to induce the cascade, whereas the alternative pathway can start it without: a fact enabling the host to react to invading pathogens even before antibodies have evolved. The alternative pathway therefore is also regarded as an early defence system of innate immunity. Ultimately resulting in the formation of C5b-C9, both pathways primarily use C3 and the subsequent activation of the opsonin C3b to induce the defence system.
Comparable to antibiotic resistance, pathogens have also evolved strategies to elude immunologic attacks. Isolates of *Enterobacteriaceae* often show “serum resistance” whereas commensal gram-negative bacteria usually are sensible. Serum resistance has been correlated with a degree of pathogenicity.
The underlying mechanism of such a phenomenon is not well known. Two common hypotheses regarding capsular polysaccharides (CPS) and O antigens have been established for *Klebsiella spp*.: The CPS are suspected to cover and mask the underlying Lipopolysaccharides (LPS) -generally activating complement- and thereby prevent immunologic attack since CPS itself is not an inductor. But O side chains of the LPS reach through the masking CPS layer of the bacterium and do activate complement. However, C3b is preferentially deposited onto the longest O-polysaccharide. Consequently the formation of the lytic membrane attack complex is too far away to damage the bacterial cell. Additionally, the smooth LPS of pathogenic strains only activate the alternative pathway of the complement cascade, whereas serum sensible bacteria usually induce both, the classical and alternative pathway. A fact resulting in fewer “bombardments” for the pathogenic strains.
The mentioned hypothesis about O antigens were the result of studies done with O1 serotypes. But for *Klebsiella spp*. eight different serotypes are currently known. Whether serum resistance applies to all subtypes can only be assumed. Even within a specific O serotype this characteristic seems not to be stable. Studies conducted with *Aeromonas hydrophilia* serotype O:34 have shown LPS composition to depend on environmental factors, namely osmolarity. LPS was found to be rough in low osmolarity and smooth in high osmolarity conditions.

Podschun and Ullman: *“ Thus , the same bacterial strain may be serum resistant at host body sites with a high-osmolarity milieu, such as the urinary tract, and serum sensitive at low-osmolarity body locations like the respiratory tract.”*

Siderophores

Functioning as a redox catalyst in proteins involved in the respiratory chain, iron is an essential factor in bacterial growth. Since it is intra- and extracellularly bound to proteins the level of free bioavailable iron is several thousand fold too low (10^{-18} M) for normal bacterial growths. Intracellularly it is bound to haemoglobin, ferritin, haemosiderin and myoglobin whereas lactoferrin and transferrin are high-affinity iron-binding proteins acting extracellularly.

To ensure their supply of the valuable element, bacteria secrete siderophores. These are high-affinity, low-molecular weight iron chelators capable of detaching iron from the host's proteins.

Enterobacteriaceae can produce two chemically different types of iron chelators. The common phenolate-type siderophore as well as the infrequently synthesised hydroxamat-type.

Enterobactin, also called enterochelin is a well phenolate-type siderophore produced by almost al clinical isolates of *E. coli* and *Salmonella spp.* It is a cyclic trimer of 2,3-dihydroxy-benzoyl-serine implying the main uptake system of *Enterobacteriae*. Because of its remarkably high affinity to iron its main target is transferring bounded iron, although the impact on virulence remains contradictory. But not for aerobactin, a hydroxamat-type siderophore which has clearly proven to increase the degree of bacterial pathogenicity. A fact applying also for *Klebsiella spp.* although they have rarely been observed to use aerobactin. While it has a considerably lower affinity to Fe(III) its great stability and high solubility make it far more effective. Furthermore aerobactin, in contrast to enterobactin directly detaches iron from the host cells and can be recycled after each turn of transport.

As it has already been mentioned *Klebsiella spp.* have rarely been observed to synthesise aerobactin itself but get more virulent if they do so. *Klebsiellae* have evolved a mechanism to take advantage of aerobactin producing bacterial competitors by expressing only aerobactin receptors.[66]

K. oxytoca cytotoxin

There have been made efforts to characterize the cytotoxin of *K. oxytoca*. HPLC-purified fraction of the culture supernatant were analyzed by means of FAB mass spectroscopy and NMR. The molecular mass of the heat-labile toxin was stated to be 217.1062 Da and the molecular formula characterized as: C8H15O4N3[58]

It has been suggested that the toxin was a nonpeptide substance. Other bacterial toxins of low molecular weight could not be related to it so far.[40]

Minami et al. reported a dose-dependent inhibition of the DNA and RNA synthesis of HEp-2 cells followed by a decrease of intracellular ATP concentration if exposed to the toxigenic culture supernatant. Protein synthesis on the contrary was observed to be only slightly inhibited.[58]

Toxic effects also were clearly demonstrated using ligated ileal loops of rabbits. Injection of the toxin caused a dose dependant fluid accumulation within 12 hours. Intense mucosal haemorrhage and erosion was observed in histological specimens taken.[56]

Minami et al.: "…results suggest that cytotoxicity toward HEp-2 cells is primarily due to the inhibitory effect of the cytotoxin on nucleic acid synthesis, possibly on DNA synthesis."[58]

Since two of the toxigenic strains investigated possessed plasmids of different sizes whereas one strain did not it was drawn the conclusion that the cytotoxin is chromosomally encoded.[58]

The data mentioned above were published some 10 years ago. Not much more insight concerning the molecular characteristics of the cytotoxin has been gained in the meanwhile. Thus molecular structure remains unknown[40]

C. difficile toxins

C. difficile is not an invasive microorganism. It does not penetrate the enterocytal layer. Damage to the colonocytes is due to processes originating from toxins produced by the pathogenic strains.

Two toxins have definitely been identified so far. A third is currently being discussed after having been recently detected.

C. difficile toxin A (cdTA), 308 kDa and toxin B (cdTB), 270 kDa are mono-chained, heat-labile proteins.[8 33 74]The toxins are being co-expressed, discharged during sporulation and don't have a signal sequence (in contrast do other bacterial toxins).[74]CdTA and B both show three ,equally structured domains. The catalytic domain is located on the N- terminus (65kDa). On the C-terminus is to be found the receptor-binding-domain. The translocation-domain is suspected to be situated in the middle of the molecule.

Binding of cdTA/B to the target receptor is assumed via carbohydrate structures because of sequence homologies of amino acids of carbohyrate domains from bacterial glycosyltranferases. Target receptors have not been found so far but it seems highly probable that the two differing toxins do not use the same receptor.

After binding to the receptor and subsequent endocytosis specific enzyme activities evolve in the cytoplasm of the affected cells. Enzymes act as mangan-dependent, mono-glycosyltransferases using the ubiquitary sugar UDP-glucose as co-substrate. Via mono-glycosylation, the Rho-proteine gets added a glucose remnant to its amino acid Threonine-37, which is located in the effector region of Rho. Glycosylation, now blocks the binding of active proteins such as kinases resulting in a complete stop of cellular signalling.

Thus depolymerisation of actin-filaments leads to the disintegration of the actin-cytoskeleton with impairment of all actin dependent functions.

The family of Rho-proteins include small GTP-binding proteins belonging to Ras-GTPases(20-23kDa) and being involved in intracellular signalling.

The small GTP-binding proteins act as molecular transducers, activated as GTPs and switched-off as GDPs. Their level of activity is modulated by other proteins. Guaninnucleotide exchange factors (GEF) catalyse the loading with GTP and thus lead to activation. Binding of GTP affects the molecular structure of the effector region (Switch 1) allowing Rho-protein to interact with so-called effector proteins.

These are serin-threonin-kinases, phospholipid kinases, phospholipases or adapter proteins which are activated by the interaction with GTP-bound Rho. Activated effector proteins evolve enzyme activity or, in case of adapter proteins recruit further regulative proteins. Rho dependent signals are simultaneously transmitted and amplified. Rho proteins are in charge of regulating the function of the actin cytoskeleton. Therefore Rho plays a key role for cellular qualities such as motility, cell to cell contacts, endo /exocytosis and morphology. *C. difficile* toxins are capable of interfering with these complex organised signal transductions. The cytotoxic effects of *C. difficile* toxin can satisfactorily be explained with the above mentioned.(2) What remains unclear is the pathophysiology of secretory diarrhoea as well as the inflammation associated. It is discussed whether the toxins may induce diarrhoea via stimulation of the enteric nerve system, comparable to cholera toxins.[74]

Toxin A

Toxin A has shown to be predominantly responsible for fluid efflux, using an animal model. CdTA, causes haemorrhagic fluid accumulation and damage to intestinal mucosa by intense inflammation.[8 33 49]Rabbid distal colon loops showed significant neutrophil infiltration and increased production of prostaglandin E_2 and leukotrienes. Though, both toxins induce proinflammatory monokines and elicit a systemic humoral respons.[8 33]

Toxin B

Toxin B, on the contrary, does not cause significant fluid efflux but is a potent cytotoxine with detrimental effects on various mammalian cell lines.[49] Although cytotoxic activity of toxin B in vitro has proven to be at least 1000 times higher, cdTA accounts for much of the symptoms in vivo models.[8 33 75]

The substances work together synergistically. For example, toxin A is known to increase the oral toxicity of toxin B.[49] Considering the fact that toxin A negative cases of CDAD have been observed, toxin B must also be capable of acting independently. But most enteropathogenic strains produce both, toxin A and B.[2]

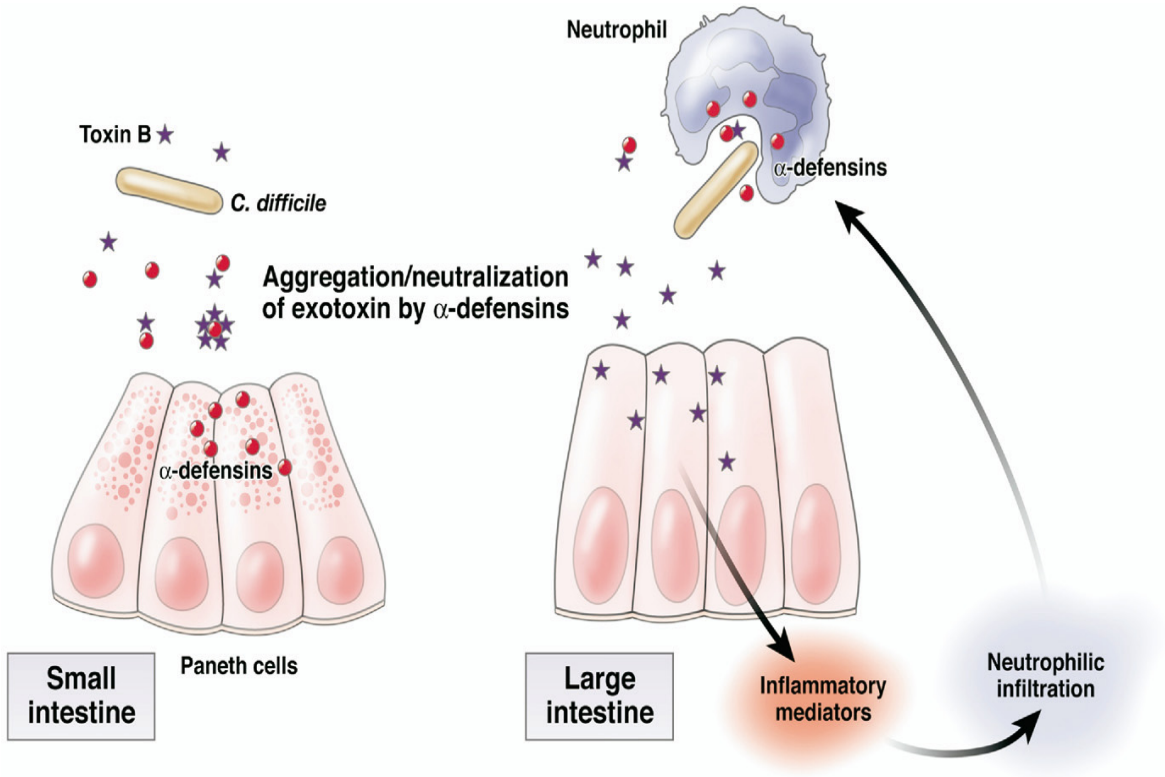
Binary toxin B

Recently a binary toxin has been discussed as potentially pathogenic. The toxin is an actin-specific ADP-ribosyltransferase. It can be isolated in 6% of *C. difficile* strains. Two genes are coding for the binary toxin. Yet the role of it in CDAD is not fully elucidated. Also it is suspected to be responsible for some severe cases of pseudomembraneous colitis.

Pathogenic *C. difficile* strains are known to have a pathogenicity locus (PaLoc) that contains the genes coding for toxin A and B. The genes coding for the binary toxin are to be found outside of this PaLoc- region.[2]

Human α-defensins: Defending against *C. difficile* toxins

Figure: 11 Defensins versus bacteria



NITA H. SALZMAN: *"A model for Toxin B-defensin interaction in the intestine. In the small intestine, high concentrations of HD5 result in Toxin B aggregation and prevention of intoxication. In the large intestine, inadequate amounts of α -defensin are present to aggregate or inhibit Toxin B, resulting in epithelial intoxication, inflammation, and neutrophil infiltration."*

Gieseman et al have provided evidence that humans α-defensins may play a protective role against cytotoxins produced by *C. difficile*. In particular *C. difficile* toxin B was proven to be inhibited by α-defensins.[27] Defensines are peptides with antibiotic activity produced by animals and plants. They are part of the innate host defence system. This finding may give some explanation for the question why not all infected individuals develop CDAD.[72] (see Figure 11)

Bacterial toxins and colitis

AAHC resembles other forms of toxin-induced colitis. *Enterohemorrhagic Escherichia coli (EHEC)* and *Shigella* species cause similar pathologic findings in histological examinations.

Alterations in AAHC were more prominent in the right colon which is comparable to findings of colitis caused by *EHEC*.[41]

It shall be commented on diseases caused by *EHEC* and *Shigella spp.* since pathologic mechanisms might be similar to those of AAHC.

If an enterotoxin is the major or exclusive pathogenic cause of acute bacterial diarrhoea it can be classified as toxigenic diarrhoea. On the contrary it will be classified as invasive if the primary event is penetration of the mucosal surface. But in the long run many invasive pathogens often produce enterotoxins as well.[33]

[33]

Shigella

Diarrhoea due to *Shigella spp.* has been described since early history. Since today there has been a constant endemic incidence in tropical and temperate zones. It is known to become more prevalent in wartime as in the American Civil War with 44.500 deaths.

Shigellae closely resemble *E. coli* and also belong to the *Enterobacteriaceae*, a group of Gram-negative enteric organisms. *Shigellae* can be divided into four different major subgroups (groups A to D). Different to *E. coli* they are non-motile,

do not produce gas from glucose and generally are lactose negative. *Shigellae* are classified as invasive pathogens.[33]

For virulence and invasion of the mucosa a plasmid is required. High fever, abdominal cramps and watery diarrhoea usually are the first signs of the disease. Watery diarrhoea is followed by mucous stools and severe tenesmus after one to two days. Frequent neurological complication include seizures, lethargy and confusion.[49]

Shigella toxins

Although invasion of the mucosal surface is a characteristic of *Shigella spp.* it also produces different toxins that play an important role in the pathogenesis of the disease.

The toxin is a potent, protein exotoxin which consists of one A subunit and five B subunits. Subunit A has a molecular weight of 32.000 Daltons whereas each of the five B subunits weigh 7.716 Daltons. The structure is very similar to toxins produced by some *E. coli* strains. These toxins were therefore named Shiga-like toxins (SLTs). The real Shiga toxin has only a single amino acid difference from SLT-I. Monoclonal antibodies against SLT-I have shown to neutralize purified Shiga toxin and antiserum against Shiga toxin on the other hand neutralizes SLT-I. During natural infection Shiga toxins are produced in sufficient amounts to elicit an immune response. The protective role of serum antitoxin antibodies remains questionable though.

After reduction of the disulfide bonds and formation of the enzymatically active A_1 fragment, subunit A evolves its biologic activity while subunit B has bound to the receptor site.

The B subunit binds to a glycolipid receptor on the intestinal microvillus membranes. It is a globotriosyl ceramide. Identical to the mechanism of *E. coli* SLT-I and SLT.II toxins, Shiga toxin inhibits protein synthesis. It does so by catalytic inactivation of the 60S ribosomal subunits. The inactivation results in cessation of polypeptide chain elongation and thus stops protein synthesis. Probably because of the ability to inhibit protein synthesis Shiga toxin causes toxic effects to selected mammalian cell lines and fluid accumulation in ligated ileal loops of rabbits. Whether Shiga toxin may provoke active fluid secretion remains

unclear. No mechanisms such as increase in cyclic nucleotides explaining active secretion could be proven.

Infection with *S. dysenteriae* sometimes was observed to cause haemolytic-uremic syndrome (HUS). Because infection with high-cytotoxin-producing *E. coli* also led to HUS it was suggested that endothelial damage caused by Shiga toxin and SLTs might be the primary event in HUS.

Apart from Shiga toxin it seems probable that also other toxins are produced by *Shigellae*.[49]

Enterohemorrhagic E. coli (EHEC)

E. coli O157:H7 was the strain associated with outbreaks of acute hemorrhagic colitis in Michigan and Oregon in 1982. It is accused of accounting for 15% to 36% of cases of hemorrhagic colitis in Canada, the United Kingdom and the USA.

The spectrum of the disease includes bloody diarrhoea in 95% of cases, HUS and thrombotic thrombocytopenic purpura. Endoscopic examination may show a friable inflamed mucosa with patchy erythema, superficial ulcerations and oedema.

EHEC strains produce at least two toxins. SLT-I and SLT-II. They cause characteristic lesions in tissue culture lines.[33]

As already mentioned SLT-I is neutralized by antibodies against Shiga toxin.

SLT-II on the contrary is not neutralized by such antibodies or monoclonal antibodies against SLT-I. Synonyms of SLT-I and SLT-II are verotoxin 1 and 2 respectively.

SLT-I was purified both from *EHEC* and from *Enteropathogenic E. coli (EPEC)* strains. As described previously, it is highly similar to Shiga toxin. SLT-II again is structurally similar to Shiga toxin and SLT-I but immunologically distinct. Structural genes have a homology of 58% with SLT-I. It has shown to consist of one A subunit but multiple B subunits with a molecular weight of 60.000 Daltons. SLT-I and SLT-II are bacteriophage encoded. Not surprisingly, Shiga-like toxins use a similar pathogenic mechanism as Shiga toxins. Biologic activity is the same: fluid secretion, cytotoxic activity and lethality in mice.

Also they share the same binding receptor. It is the globotriasyl ceramide (Gb_3) a glycosphingolipid. By inhibiting elongation factor 1-dependend binding of

aminoacyl-tRNA to ribosomes they inactivate the 60S ribosomal subunit resulting in loss of protein synthesis. On eukaryotic ribosomes SLT-II acts on the same molecular site as Shiga toxin.[33 33]

Other bacteria less frequently causing AAD

Clostridium perfringens

C. perfringens is a gram-positive, obligate anaerobe, non-motile, encapsulated, spore-forming rod. It can withstand exposure to oxygen for up to 72 hours, making it remarkably aerotolerant compared to other species like *C. difficile* for example. On the basis of serologic and biochemical characteristics the bacterium can be subclassified into 5 major types. Mainly toxin A and C are responsible for human diseases.[33 33 38]

Food poisoning is caused by toxin A. This enterotoxin is a heat-labile protein consisting of a single polypeptide chain with a molecular weight of about 34.000 daltons.[33 33 49]Usually associated with disease are type A strains but type C and D strains have also been reported to cause symptoms.[49 87] The production of the enterotoxin probably is plasmid-mediated and does only occur during sporulation of the cells. It has been suggested that it might be a precursor molecule for a spore coat component. During natural infection it had not shown to cause a systemic immune response. Specific antiserum though can neutralize the biologic activities of the toxin. Animal loop models have shown that *C. perfringens* enterotoxin causes fluid secretion with the highest activity in the ileum. Although a specific receptor could not be found it is suggested that it binds on a high molecular weight protein on the intestinal brush border. The cellular mode of action is not clear but direct effects on cell membrane with modulation of the fluid transport seem to be likely. Functional and structural changes of intestinal cell membranes have been demonstrated.[49]It has also been reported that the toxin can

inhibit glucose transport and damage the intestinal epithelium and thus lead to protein loss.[33] [33] A very high bacterial count of 10^6 per gram ingested food is required to cause disease.[38] Epidemics are characterised by high attack rates with a rather short incubation time between 8 and 22 hours. Outbreaks predominantly occur after large gatherings. Meals, mainly beef, turkey and chicken are precooked and reheated for such purposes, allowing the surviving spores to germinate in the meanwhile. After one outbreak the toxins were demonstrated in the stool of the sick but not of the healthy individuals.[49]

Usually no vomiting, fever or other signs of infection but severe abdominal cramps and watery diarrhoea characterise the clinical feature. Symptoms resolve spontaneously in 24 to 36 hours. Specific treatment is therefore usually not required.[33] [33]

Enteritis necroticans

Enteritis necroticans is a severe form of infection with *C. perfringens* toxin C. Also known as “Darmbrand” or pigbel, it is associated with a mortality rate of approximately 40%. The disease has first been described in post-World War II where people consumed rancid meat. The term Pigbel refers to orgiastic consumptions of poorly cooked pork during native feasts in New Guinea. Large amounts of meat were used to be eaten over a time of three to four days. To result in infection, quantities of inappropriately cooked food, especially meat, need to be ingested. The symptoms involve intense abdominal pain, vomiting as well as bloody diarrhoea and shock after an incubation period of 24 hours. The high mortality usually results from intestinal perforation.[33] [33]

Staphylococcus aureus

The association with AAD and *Staph. aureus* is rather low. Surprisingly though is the report of 60 *Staph. aureus* AAD cases which could recently be identified by a 2- year prospective study conducted in France.[4]

Coagulase-positive strains of *Staph. aureus* are responsible for the typical gastrointestinal affections. The enterotoxins A, B, C, D, and E have been regarded to be accountable for food poisoning. In contrast to *C. perfringens* toxins they are

heat-resistant peptides. These single polypeptide chains cause secretion of water and electrolytes in a rat intestinal loop model.

Human volunteers additionally showed vomiting. Many types of meals can be contaminated with pathogenic *Staph. aureus* strains but the bacterium tends to favour foods with high salt or sugar concentrations. Such foods include ham, canned meat, custard or cream.

After a short incubation period of approximately 3 hours, first symptoms will develop. It shows a high attack rate and was observed to be usually clustered within a group of people such as families. If the toxin concentration was high an attack rate of 100% has been reported, though a varying dose response among individual volunteers was shown.

Pathogenic mechanisms of infection with *Staph. aureus* are obvious. First, the pathogenic, enterotoxin-producing strains need to get in contact with foods.[33 33] The major mode of transmission is the human hand. Food workers carrying the bacterium, usually are the source of contamination.[38] The edibles have mostly been sliced, cut, ground or mixed by *Staph. aureus* pos. labours. After contamination the bacterium needs suitable growth conditions, sufficient time and temperature to grow.

Initial and usually profuse symptoms are nausea and vomiting, followed by abdominal cramps and often diarrhoea. Fever is uncommon but elevated temperature has been observed in severe cases. Vomiting can be very strong and lead to metabolic alkalosis. Death is very unusual but hypotension and marked prostration has rarely been documented. Symptoms usually resolve within 24 to 48 hours.

The physician is normally not contacted by people suffering from *Staph. aureus* food poisoning. Only severely affected individuals may require supportive care. A specific therapy is not available. Rehydration and correction of alkalosis can be considered.[33 33]

Salmonella spp.

All *Salmonellae* belong to the species *Salmonella enterica* and are part of the family of *Enterobacteriaceae*. They are differentiated into subgenera and numerous serovars.

Subgenus I comprises the major types responsible for human infections.[38] *Salmonellae* are Gram-negative, predominantly motile bacilli.

Four clinical syndromes are known to be caused by *Salmonella* infection:

Table12: Clinical syndromes known to be caused by *Salmonella*	
75%	gastroenteritis
5-10%	bacteraemia, w/o gastrointestinal involvement
5-10%	typhoidal ("enteric") fever
5%	localized infection[38]

Information from reference:[33 33]

Association with antibiotics and gastroenteritis has been reported. According to a study conducted in 1984, 12 out of 18 patients infected with multi-resistant *Salmonella newport* have received penicillin derivatives before onset of diarrhoea.[42]

Several toxins are produced by *Salmonellae*. Their role in the pathogenesis of the disease is poorly understood though. It was suggested that they have to be understood as co-factors to other pathologic mechanisms.[49]

The major route of transmission is by "5 Fs": faeces, fingers, flies, fomites and food. The bacterium is ubiquitous in the environment. Nonhuman reservoirs such as animals or animal products seem to play a crucial role in the mode of transmission. Almost 50 % of 500 outbreaks were related to animals. Most frequently, meats, poultry and eggs were involved.

Poultry, pigs and cattle are known to be contaminated most heavily among many other creatures. Although colonised most animals cohabit rather peacefully with the bacteria and do not get ill. A vertical transmission from the chicken to the egg via the transovarian route is possible.

Commercially prepared foods, such as chocolate balls have been responsible for multi national outbreaks. Infections have been increasing in incidence in the last years. Again, eggs and poultry were held responsible.

In contrast to many other pathogens causing diarrhoea *Salmonella* preferentially attacks the ileum and to a lesser extent the colon. The bacterium rapidly makes its way through the epithelial surface and reaches the lymphatics and blood stream. About 10^7 organisms need to be inoculated to result in infection in 50 % of human volunteers.

Children younger than one year and the elderly are most susceptible for *Salmonella* infection. It seems probable that this is due to immunologic immaturity or impairment, respectively.
Although humoral antibody titers can reach impressive levels, association with severity or mildness of disease could not be observed.
As already mentioned, correlation with antibiotic treatment prior to the symptomatic onset of *Salmonella* infection has been stated in literature. Animal models substantiate such observations. By pre-treating the animals with antibiotic agents a lower bacterial count is necessary to cause infection. Reduced gastric acid, a mechanism discussed earlier also seems to increase susceptibility for the pathogen.

Clinical features most commonly involve gastroenteritis. Followed by abdominal cramps and diarrhoea the disease usually commences with nausea and vomiting. The onset of symptoms has been observed to vary usually between 6 to 48 hours after contact. But symptom free periods of 12 days have also been described. In 50 % of subjects fever is accompanying the disease. Diarrhoea typically resolves in 3 to 4 days. A few loose stools as well as grossly bloody and purulent faeces can be observed in *Salmonella* gastroenteritis. Also a cholera-like syndrome has been described in achlorhydric patients.
Almost all organs can get affected after the bacterium has invaded the bloodstream. Persistent fever is an indicator suggesting bacteraemia or focal infection.[33 33]

Salmonella Colitis

Colonic involvement can be the predominant aspect of *Salmonella* infection in some patients. Although not typical of the disease such course can dominate the clinical picture. Toxic megacolon and perforation also can occur. In contrast to normal *Salmonella* gastroenteritis with diarrhoea for 5 days, colonic affection tends to present with protracted symptoms for 10 to 15 days. Clinical signs can be confused with the picture of idiopathic ulcerative colitis. Nearly one half of patients egest grossly bloody faeces. Findings in sigmoidoscopy and rectal biopsy may also resemble chronic inflammatory processes. *Salmonellae* often already have disappeared when stools are examined. This is a fact that makes it almost

impossible to distinguish between the diseases. *Salmonella* colitis can even last for more than two months, but the average duration is three weeks. The good message is that patients can be assured of the self- limited course of the infection.
Patients with no history of colitis and an acute onset of diarrhoea lasting for up to three weeks should be suspected to suffer from infectious colitis. Pathogens to be considered are *Salmonella*, *EHEC*, *Shigella, Campylobacte*r and *C. difficile*.
Infectious causes should be detected because inappropriate administration of glucocorticoids could result in silent perforation and septicaemia.[33 33]

Treatment

Many antibiotics have been used to treat *Salmonella* gastroenteritis but did not show to improve clinical state. 12 randomized trials have been reviewed to question whether antibiotics could alter the rate of patients' recovery or not. No differences to those treated with placebo were found. In fact relapses were more common in patients who had received antimicrobial agents. Therefore antibiotics should not be used in common *Salmonella* gastroenteritis.
Excluded from this rule are patients suffering from severe co morbidities or who are developing sings of sepsis. If antimicrobial therapy is not limited to such high risk patients and based on sensitivity testing, levels of drug resistance will further increase.[33 33]

Conclusion

With particular emphasis on bacterial infection, different causes of antibiotic-associated diarrhoea have been reviewed so far. Literature on several pathogens suspected to be responsible for AAD has delivered comprehensive information on this topic.

Because of its rising relevance due to increasing frequency and mortality rate *C. difficile* has received special attention. Also reviewed was literature on *K. oxytoca* recently identified to be responsible for AAHC. But knowledge about the processes resulting in symptomatic disease remains vague.

K. oxytoca and AAHC

AAHC is a relatively rare cause of AAD. At the university clinic of Graz, Austria, only 5 five cases were reported during an observation period of 38 months. Compared with 121 cases of infection with *C. difficile*, 71 cases of infection with *Salmonella* and 54 cases of infection with *Campylobacter* were known. Thus *K. oxytoca* does not represent a frequent cause of diarrhoea. But incidence was higher than of *Shigella* (2 cases) and *Yersinia* or *Enterohemorrhagic E. coli* (none). The hospital admits approximately 10.000 patients a year. Prevalence of healthy individuals colonised with *K. oxytoca* amounted to 1.6% whereas in France a prevalence of 9% was reported.[41]

Typically, patients suffering AAHC are young and otherwise healthy.[40] Therefore, prevalence is suspected to be higher outside the hospital setting. It seems probable that family physicians see more patients with AAD due to *K. oxytoca*. Since symptoms resolve spontaneously after cessation of antibiotics (meantime 4 days) the disease might be underdiagnosed.[41] As noted in two case reports, the picture of AAHC has also been observed to develop without anamnesis of prior antibiotic treatment. If *K. oxytoca* might be pathogenic even in the absence of antibiotic substances is therefore been discussed.[41]

Weakened colonisation resistance irrespective of antibiotics may play an important role in such cases. (personal assumption)

Evidence for *K. oxytoca* to be the causal pathogen of AAHC has lately been supplied. Not much is know yet about the underlying pathophysiological mechanisms. But it has been suggested that this disease might be toxin induced.

Other forms of toxin-induced colitis resemble findings of AAHC. *Enterohemorrhagic E. coli* and *Shigella*, for example, have presented similar pathologic characteristics in histological examinations. Findings also are similar to those of ischemic colitis. Hypothesis of a cytotoxin mediated disease is also substantiated by the observation that crypt abscesses were absent in both humans and rats. Such abscesses are common in patients suffering infectious colitis due to invasive bacteria. Interestingly, pathologies were located in the right colon identical to findings with *Enterohemorrhagic E. coli*.

It can be concluded that detailed insight into the pathophysiology of AAHC remains vague although knowledge has considerably grown. Though the toxin itself-suggested to be the causal factor of the disease could yet not be identified.[40] More efforts will have to be made to characterize its molecular structure and thus taking a great leap forward.

C. difficile and CDAD

C. difficile has already been made responsible to cause AAD in the 1970ies. In Austria around 1400 cases of "enterocolitis due to *C. difficile"* are being treated annually. For approximately 80 individuals this disease will end lethal!

C. difficile belongs to one the few pathogens forming spores. Spores are inactive, highly resistant forms of the bacterium which usually survive routine hospital disinfection.[23] They can persist up to 40 days in the hospital environment. Spores are excreted with the faeces and are thus spread in the vicinity or the affected. Ingestion of spores may result in infection, especially in weakened individuals. The disease is mainly affecting the elderly. Individuals older than 65 have a 10-fold higher risk of getting infected compared to others.[74]

Treatment with antibiotics is the most common and therefore most relevant risk factor concerning preventive measures. Antibiotics may alter the physiologic bowel flora and thus allow pathogenic bacteria to cause disease.

C. difficile associated diseases are currently one of the most relevant nosocomial infections in European countries. Numbers of lethal cases in England were double as high as to MRSA in year 2003. The incidence of CDAD has notably risen in the last five years. But also severity, risk of recurrence and lethality of the disease clearly have increased.[2]

Due to the intense extra work necessary for CDAD cases the disease also causes considerable financial expenses to the health care system. [2]

But compared to AAHC much is known already about the pathophysiological mechanisms of CDAD. Important insight into the transmission has been gained allowing health care institutions to take preventive measures. Also the high significance of the disease for public health questions is broadly accepted.

Nevertheless CDAD still is associated with high mortality rates and treatment options for severe cases remain scarce.

References

1. Abdel-Hady H, Hawas S, El-Daker M, El-Kady R. Extended-spectrum beta-lactamase producing Klebsiella pneumoniae in neonatal intensive care unit. J Perinatol 2008.

2. AGES ÖAfGuEG. Prävention und Kontrolle von Clostridium difficile in Krankenhäusern und Einrichtungen der stationären Pflege. 2007. 152 pages AGES, Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH, Wien.

3. Altindis M, Usluer S, Ciftci H, Tunc N, Cetinkaya Z, Aktepe OC. Investigation of the presence of Clostridium difficile in antibiotic associated diarrhea patients by culture and toxin detection methods. Mikrobiyol Bul 2007; 41(1):29-37.

4. Asha NJ, Tompkins D, Wilcox MH. Comparative analysis of prevalence, risk factors, and molecular epidemiology of antibiotic-associated diarrhea due to Clostridium difficile, Clostridium perfringens, and Staphylococcus aureus . J Clin Microbiol 2006; 44(8):2785-91.

5. Ayyagari A, Agarwal J, Garg A. Antibiotic associated diarrhoea: Infectious causes. Indian J Med Microbiol 2003; 21(1):6-11.

6. Bartlett JG. Historical perspectives on studies of Clostridium difficile and C. difficile infection. Clin Infect Dis 2008; 46 Suppl 1:S4-11.

7. Bartlett JG, Gerding DN. Clinical recognition and diagnosis of Clostridium difficile infection. Clin Infect Dis 2008; 46 Suppl 1:S12-8.

8. Bartlett JG. Pseudomembranous enterocolitis and antibiotic-associated diarrhea. Feldman M, Friedman LS, Sleisenger MH. Sleisenger & Fordtran's gastrointestinal and liver disease : pathophysiology, diagnosis, management. 7th ed edition. Philadelphia : Saunders, 2002: 1914-31.

9. Beaugerie L, Metz M, Barbut F *et al*. Klebsiella oxytoca as an agent of antibiotic-associated hemorrhagic colitis . Clin Gastroenterol Hepatol 2003; 1(5):370-6.

10. Beaugerie L, Petit JC. Microbial-gut interactions in health and disease. Antibiotic-associated diarrhoea. Best Pract Res Clin Gastroenterol 2004; 18(2):337-52.

11. Boyce JM, Havill NL. Nosocomial antibiotic-associated diarrhea associated with enterotoxin-producing strains of methicillin-resistant Staphylococcus aureus. Am J Gastroenterol 2005; 100(8):1828-34.

12. Byrn JC, Maun DC, Gingold DS, Baril DT, Ozao JJ, Divino CM. Predictors of mortality after colectomy for fulminant Clostridium difficile colitis. Arch Surg 2008; 143(2):150-4; discussion 155.

13. Cadle RM, Mansouri MD, Logan N, Kudva DR, Musher DM. Association of proton-pump inhibitors with outcomes in Clostridium difficile colitis. Am J Health Syst Pharm 2007; 64(22):2359-63.

14. Cai J, Wang Z, Cai C, Zhou Y. Characterization and identification of virulent Klebsiella oxytoca isolated from abalone (Haliotis diversicolor supertexta) postlarvae with mass mortality in Fujian, China. J Invertebr Pathol 2008; 97(1):70-5.

15. Calhoun JH, Murray CK, Manring MM. Multidrug-resistant organisms in military wounds from Iraq and Afghanistan. Clin Orthop Relat Res 2008; 466(6):1356-62.

16. Camacho N, Espinoza C, Rodriguez C, Rodriguez E. Isolates of Clostridium perfringens recovered from Costa Rican patients with antibiotic-associated diarrhoea are mostly enterotoxin-negative and susceptible to first-choice antimicrobials. J Med Microbiol 2008; 57(Pt 3):343-7.

17. Clabots CR, Johnson S, Olson MM, Peterson LR, Gerding DN. Acquisition of Clostridium difficile by hospitalized patients: evidence for colonized new admissions as a source of infection. J Infect Dis 1992; 166(3):561-7.

18. Dial S, Delaney JA, Barkun AN, Suissa S. Use of gastric acid-suppressive agents and the risk of community-acquired Clostridium difficile-associated disease. JAMA 2005; 294(23):2989-95.

19. Etienney I, Beaugerie L, Viboud C, Flahault A. Non-steroidal anti-inflammatory drugs as a risk factor for acute diarrhoea: a case crossover study. Gut 2003; 52(2):260-3.

20. Evans JM, McMahon AD, Murray FE, McDevitt DG, MacDonald TM. Non-steroidal anti-inflammatory drugs are associated with emergency admission to hospital for colitis due to inflammatory bowel disease. Gut 1997; 40(5):619-22.

21. Fisher DJ, Miyamoto K, Harrison B, Akimoto S, Sarker MR, McClane BA. Association of beta2 toxin production with Clostridium perfringens type A human gastrointestinal disease isolates carrying a plasmid enterotoxin gene. Mol Microbiol 2005; 56(3):747-62.

22. Flemming K, Ackermann G. Prevalence of enterotoxin producing Staphylococcus aureus in stools of patients with nosocomial diarrhea. Infection 2007; 35(5):356-8.

23. Fordtran JS. Colitis due to Clostridium difficile toxins: underdiagnosed, highly virulent, and nosocomial. Proc (Bayl Univ Med Cent) 2006; 19(1):3-12.

24. Gekle M. Funktion des Magen-Darm-Trakts, Energiehaushalt und Ernährung. Klinke R, Pape H-C, Silbernagl S . Physiologie. 5 edition. Stuttgard: Thieme, 2005: 407-83.

25. Gerding DN, Johnson S, Peterson LR, Mulligan ME, Silva J Jr. Clostridium difficile-associated diarrhea and colitis. Infect Control Hosp Epidemiol 1995; 16(8):459-77.

26. Gerding DN, Muto CA, Owens RC Jr. Measures to control and prevent Clostridium difficile infection. Clin Infect Dis 2008; 46 Suppl 1:S43-9.

27. Giesemann T, Guttenberg G, Aktories K. Human alpha-defensins inhibit Clostridium difficile toxin B. Gastroenterology 2008; 134(7):2049-58.

28. Goorhuis A, Debast SB, van Leengoed LA *et al*. Clostridium difficile PCR ribotype 078: an emerging strain in humans and in pigs? J Clin Microbiol 2008; 46(3):1157; author reply 1158.

29. Gould PC, Khawaja FI, Rosenthal WS. Antibiotic-associated hemorrhagic colitis. Am J Gastroenterol 1982; 77(7):491-3.

30. Granier SA, Plaisance L, Leflon-Guibout V *et al*. Recognition of two genetic groups in the Klebsiella oxytoca taxon on the basis of chromosomal beta-lactamase and housekeeping gene sequences as well as ERIC-1 R PCR typing. Int J Syst Evol Microbiol 2003; 53(Pt 3):661-8.

31. Guarner F, Malagelada JR. Gut flora in health and disease. Lancet 2003; 361(9356):512-9.

32. Hall JF, Berger D. Outcome of colectomy for Clostridium difficile colitis: a plea for early surgical management. Am J Surg 2008.

33. Hamer DH, Gorbach SL. Infectious diarrhea and bacterial food poisoning. Feldman M, Friedman LS, Sleisenger MH. Sleisenger & Fordtran's gastrointestinal and liver disease : pathophysiology, diagnosis, management. 7th ed edition. Philadelphia : Saunders, 2002: 1864-911.

34. Heer M, Sulser H, Hany A. Segmental hemorrhagic colitis following amoxicillin therapy. Schweiz Med Wochenschr 1989; 119(21):733-5.

35. Hickson M, D'Souza AL, Muthu N *et al*. Use of probiotic Lactobacillus preparation to prevent diarrhoea associated with antibiotics: randomised double blind placebo controlled trial. BMJ 2007; 335(7610):80.

36. Higaki M, Chida T, Takano H, Nakaya R. Cytotoxic component(s) of Klebsiella oxytoca on HEp-2 cells. Microbiol Immunol 1990; 34(2):147-51.

37. Highsmith AK, Jarvis WR. Klebsiella pneumoniae: selected virulence factors that contribute to pathogenicity. Infect Control 1985; 6(2):75-7.

38. Hof H, Rüdiger Dörries. Medizinische Mikrobiologie, Duale Reihe. 3. edition. Stuttgart: Thieme, 2004.

39. Hogenauer C, Hammer HF, Krejs GJ, Reisinger EC. Mechanisms and management of antibiotic-associated diarrhea. Clin Infect Dis 1998; 27(4):702-10.

40. Hogenauer C, Hinterleitner T. Klebsiella oxytoca as a cause of antibiotic-associated colitis. Scheld WM, Hammer SM, Hughes JM. Emerging Infections 8. in press edition.

41. Hogenauer C, Langner C, Beubler E *et al*. Klebsiella oxytoca as a causative organism of antibiotic-associated hemorrhagic colitis. N Engl J Med 2006; 355(23):2418-26.

42. Holmberg SD, Osterholm MT, Senger KA, Cohen ML. Drug-resistant Salmonella from animals fed antimicrobials. N Engl J Med 1984; 311(10):617-22.

43. Iregbu KC, Anwaal U. Extended spectrum Beta-Lactamase-producing Klebsiella pneumoniae septicaemia outbreak in the Neonatal Intensive Care Unit of a tertiary hospital in Nigeria. Afr J Med Med Sci 2007; 36(3):225-8.

44. Kim JY, Sohn JW, Park DW, Yoon YK, Kim YM, Kim MJ. Control of extended-spectrum {beta}-lactamase-producing Klebsiella pneumoniae using a computer-assisted management program to restrict third-generation cephalosporin use. J Antimicrob Chemother 2008.

45. Kishida T, Sato J, Fujimori S *et al*. An endoscopic study of antibiotic-associated hemorrhagic colitis. Nippon Ika Daigaku Zasshi 1992; 59(6):450-6.

46. Koga H, Aoyagi K, Yoshimura R, Kimura Y, Iida M, Fujishima M. Can quinolones cause hemorrhagic colitis of late onset? Report of three cases. Dis Colon Rectum 1999; 42(11):1502-4.

47. Kokkotou E, Moss AC, Michos A *et al*. Comparative efficacies of rifaximin and vancomycin for treatment of Clostridium difficile-associated diarrhea and prevention of disease recurrence in hamsters. Antimicrob Agents Chemother 2008; 52(3):1121-6.

48. Krause R, Reisinger EC. Candida and antibiotic-associated diarrhoea. Clin Microbiol Infect 2005; 11(1):1-2.

49. Lebenthal E, Duffey ME. Textbook of secretory diarrhea. New York : Raven Press, 1990.

50. Louie TJ, Peppe J, Watt CK *et al*. Tolevamer, a novel nonantibiotic polymer, compared with vancomycin in the treatment of mild to moderately severe Clostridium difficile-associated diarrhea. Clin Infect Dis 2006; 43(4):411-20.

51. Lund-Tonnesen S , Berstad A, Schreiner A, Midtvedt T. Clostridium difficile-associated diarrhea treated with homologous feces. Tidsskr Nor Laegeforen 1998; 118(7):1027-30.

52. Marchaim D, Zaidenstein R, Lazarovitch T, Karpuch Y, Ziv T, Weinberger M. Epidemiology of bacteremia episodes in a single center: increase in Gram-negative isolates, antibiotics resistance, and patient age. Eur J Clin Microbiol Infect Dis 2008.

53. Marcos LA, DuPont HL. Advances in defining etiology and new therapeutic approaches in acute diarrhea. J Infect 2007; 55(5):385-93.

54. McFarland LV, Mulligan ME, Kwok RY, Stamm WE. Nosocomial acquisition of Clostridium difficile infection. N Engl J Med 1989; 320(4):204-10.

55. Miendje Deyi VY , Vandenberg O, Mascart G *et al*. Diagnostic value of five commercial tests for the rapid diagnosis of Clostridium difficile-associated disease. Clin Lab 2008; 54(1-2):9-13.

56. Minami J, Katayama S, Matsushita O, Sakamoto H, Okabe A. Enterotoxic activity of Klebsiella oxytoca cytotoxin in rabbit intestinal loops. Infect Immun 1994; 62(1):172-7.

57. Minami J, Okabe A, Shiode J, Hayashi H. Production of a unique cytotoxin by Klebsiella oxytoca. Microb Pathog 1989; 7(3):203-11.

58. Minami J, Saito S, Yoshida T, Uemura T, Okabe A. Biological activities and chemical composition of a cytotoxin of Klebsiella oxytoca. J Gen Microbiol 1992; 138(9):1921-7.

59. Missaghi B, Valenti AJ, Owens RC Jr. Clostridium difficile Infection: A Critical Overview. Curr Infect Dis Rep 2008; 10(3):165-73.

60. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983; 65(1-2):55-63.

61. Nelson R. Antibiotic treatment for Clostridium difficile-associated diarrhea in adults. Cochrane Database Syst Rev 2007; (3): CD004610.

62. Noren T, Akerlund T, Wullt M, Burman LG, Unemo M. Mutations in fusA associated with posttherapy fusidic acid resistance in Clostridium difficile. Antimicrob Agents Chemother 2007; 51(5):1840-3.

63. Noren T, Wullt M, Akerlund T, Back E, Odenholt I, Burman LG. Frequent emergence of resistance in Clostridium difficile during treatment of C. difficile-associated diarrhea with fusidic acid. Antimicrob Agents Chemother 2006; 50(9):3028-32.

64. Pepin J. Vancomycin for the treatment of Clostridium difficile Infection: for whom is this expensive bullet really magic? Clin Infect Dis 2008; 46(10):1493-8.

65. Pillai A, Nelson R. Probiotics for treatment of Clostridium difficile-associated colitis in adults. Cochrane Database Syst Rev 2008; (1):CD004611.

66. Podschun R, Ullmann U. Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev 1998; 11(4):589-603.

67. Pop-Vicas A, Neill MA. Clostridium difficile: the increasingly difficult pathogen. Crit Care 2008; 12(1):114.

68. Redelings MD, Sorvillo F, Mascola L. Increase in Clostridium difficile-related mortality rates, United States, 1999-2004. Emerg Infect Dis 2007; 13(9):1417-9.

69. Reisinger EC, Lademann M, Krause R. Antibiotic-associated diarrhea. Dtsch Med Wochenschr 2004; 129 Suppl 2:S111-3.

70. Riordan SM, McIver CJ, Wakefield D, Duncombe VM, Thomas MC, Bolin TD. Small intestinal mucosal immunity and morphometry in luminal overgrowth of indigenous gut flora. Am J Gastroenterol 2001; 96(2):494-500.

71. Rocha Ramirez JL, Parrado Montano JW, Rojas Illanes M, Sierra Montenegro E, Soto Quirino R, Manuel Rivero J. Intestinal damage by non-steroidal anti-inflammatory drugs. Rev Gastroenterol Mex 2007; 72(1):43-6.

72. Salzman NH. Defensins versus bacteria: not just antibiotics anymore. Gastroenterology 2008; 134(7):2174-7.

73. Sanchez-Hurtado K, Corretge M, Mutlu E, McIlhagger R, Starr JM, Poxton IR. Systemic antibody response to Clostridium difficile in colonized patients with and without symptoms and matched controls. J Med Microbiol 2008; 57(Pt 6):717-24.

74. Schroder O, Gerhard R, Stein J. Antibiotic-associated diarrhea. Z Gastroenterol 2006; 44(2):193-204.

75. Schroeder MS. Clostridium difficile--associated diarrhea. Am Fam Physician 2005; 71(5):921-8.

76. Schwaber MJ, Klarfeld-Lidji S, Navon-Venezia S, Schwartz D, Leavitt A, Carmeli Y. Predictors of carbapenem-resistant Klebsiella pneumoniae acquisition among hospitalized adults and effect of acquisition on mortality. Antimicrob Agents Chemother 2008; 52(3):1028-33.

77. Seidler RJ, Morrow JE, Bagley ST. Klebsielleae in drinking water emanating from redwood tanks. Appl Environ Microbiol 1977; 33(4):893-900.

78. Siegenthaler W, Blum HE. Klinische Pathophysiologie. 9. Auflage edition. Stuttgard, New York: Thieme, Georg, 2006.

79. Simango C, Mwakurudza S. Clostridium difficile in broiler chickens sold at market places in Zimbabwe and their antimicrobial susceptibility. Int J Food Microbiol 2008; 124(3):268-70.

80. Siproudhis L, Mahmoud H, Briand N *et al*. Causal assessment of drug-induced acute colitis. A prospective study of 58 consecutive cases. Gastroenterol Clin Biol 1998; 22(10):778-84.

81. Sloan LM, Duresko BJ, Gustafson DR, Rosenblatt JE. Comparison of real-time PCR for detection of the tcdC gene with four toxin immunoassays and culture in diagnosis of Clostridium difficile infection. J Clin Microbiol 2008; 46(6):1996-2001.

82. Struelens MJ, Maas A, Nonhoff C *et al*. Control of nosocomial transmission of Clostridium difficile based on sporadic case surveillance. Am J Med 1991; 91(3B):138S-44S.

83. Thompson I. Clostridium difficile-associated disease: update and focus on non-antibiotic strategies. Age Ageing 2008; 37(1):14-8.

84. Toffler RB, Pingoud EG, Burrell MI. Acute colitis related to penicillin and penicillin derivatives. Lancet 1978; 2(8092 Pt 1):707-9.

85. Vedantam G, Hecht DW. Antibiotics and anaerobes of gut origin. Curr Opin Microbiol 2003; 6(5):457-61.

86. Vonberg RP, Kuijper EJ, Wilcox MH *et al*. Infection control measures to limit the spread of Clostridium difficile. Clin Microbiol Infect 2008; 14 Suppl 5:2-20.

87. Wen Q, McClane BA. Detection of enterotoxigenic Clostridium perfringens type A isolates in American retail foods. Appl Environ Microbiol 2004; 70(5):2685-91.

List of abbreviations	
AAD	Antibiotic-associated diarrhoea
AAHC	Antibiotic-associated haemorrhagic colitis
CDAD	C. difficile-associated diarrhoea
CdT	C. difficile toxin
CRP	C-reactive protein
ESBL	Extended spectrum beta lactamase
FCS	fetal bovine serum
MEM	minimal essential medium)
MRSA	Methicillin-resistant Staphylococcus aureus
MTT	A standard colorimetric assay
NSAID	Non-steroidal anti-inflammatory drugs
PBS	Phosphate buffered saline
PMC	Pseudomembraneous colitis
SCFA	Short chain fatty acids
SLT	Shiga-like toxin
TSB	Tryptic soy broth

Curriculum vitae

Personal information

Full name	**Ulbl Florian**
Address	Rotmoosweg 31/d, 8045, Graz, Austria
Telephone	++43 650 48 52 185
E-mail	fulbl@gmx.at

Date of birth	20th September, 1981
Languages	German (mother tongue) English, Spanish, Norwegian, Swedish, French

Work experience

January 08 -June 08	Medical studies and internship in Tromsø, Norway
August/September 06	Internship in Ifakara, Tanzania, East-Africa
February 06	Medical Training in Surgery, "Diakonissen-Krankenhaus", Schladming, Austria
August 05	Medical Training in Intensive Care, "Landeskrankenhaus- Graz", Austria,
July 05	Medical Training in Intensive Care and Emergency Medicine "Virgen del Rocio", Spain
July / August 04	Medical Training in Internal Medicine, "Landeskrankenhaus bad Ausse", Austria
August /September 02	Internship at an health care facility of the trade union in Guatemala-City, Guatemala
October 01	Social Service at the Red Cross Ambulance Service, (in place of Military Service)

Education

January 08 -June 08	Medical studies at the Medical Faculty at the University of Tromsø, Norway
Since October 02	Medical studies, Medical University of Graz
September 02	End of Social Service
June 01	Higher School Certificate, Bundesrealgymnasium "BORG- Hasnerplatz", Graz
September 88 -July 98	Student at the Freien Waldorfschule Graz (Steiner School of Graz)
August 98 -February 99	Student at the Korowal- School, Leura, Blue Mountains, N.S.W., Australia

Languages

	Understanding		Oral fluency		Writing
	Hearing	Reading	Participating in a conversation	Fluency	
English	Excellent	Excellent	Excellent	Excellent	Excellent
Spanish	Very good	Very good	Very good	Very good	Very good
Norwegian	Good	Good	Good	Good	Good
Swedish	Good	Good	Good	Good	Good
French	Basic knowledge	Basic knowledge	Basic knowledge	Basic knowledge	Basic knowledge
Latin	Basic knowledge	Basic knowledge			Basic knowledge