Groundwater: hidden treasure or Pandora's box?

A case study on resistant bacteria in the drink watersupply at Oasen, Gouda,

an interdisciplinary approach

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Contents

Introduction

It is commonly accepted that the human body is covered from head to toe in bacteria. Most of these bacteria are harmless or are in fact serving us. However, in earlier times, pathogen bacteria were considered mass-killers, causing disease, illness and eventually death. This all changed in 1929, when a 15-line article by Alexander Fleming reported the discovery of penicillin. A decade later penicillin was established as a wonder drug. Life before penicillin is now hardly conceivable as bacterial infections are only rarely life threatening. The number of lives saved by penicillin runs in the millions, if not more.[1] Not long after the clinical introduction of penicillin, Fleming warned in an interview in *The New York Times* that the misuse of penicillin could lead to resistance in bacteria.[2] It was Fleming himself who warned for the shortcomings of his wonder drug. Unfortunately, the colossal demand for antibiotics and the lack of responsibility by medical practitioners has lead to misuse and bacterial resistance is now the order of the day.

Large-scale usage of antibiotics all over the world has its repercussions on the environment. As resistant bacteria take over the aquatic system, they will get incorporated into fruit and vegetables and contaminate potable water supplies, particularly in developing countries.[3] The fear of resistant bacteria is starting to show even in Europe. In the summer of 2011, people got ill in Germany, after eating cucumbers from Spain. Health investigators blamed the bacteria *E. coli*., a bacterium that is in itself usually harmless, but which has several mutant forms that are pathogenic and resistant to most forms of antibiotics.[4]

As increasing evidence of the march of the antimicrobial resistant bacteria increased awareness, governments and health agencies have started investigations on how to minimize risks for public health. These studies revealed the large-scale usage of antibiotics and its effects on water-collection areas.[5] In this study, we will try to provide a broader and more comprehensive answer to the question to what extent resistant bacteria in the aquatic system pose a threat to public health by contaminating potable water in water-collection areas. We will focus on one particular water treatment plant, namely Oasen in Gouda. Therefore, this study is focussed on one single aquatic system. Furthermore, we will narrow

[1] Elizabeth H. Oakes, *A to Z of STS scientists*, (New York: Facts on File, 2002), 97-98
[2] Camille Georges Wermuth, *The practice of medicinal chemistry*, (London: Elsevier Ltd, 2008), 19
[3] Nick G.H. Taylor et al., “Aquatic systems: maintaining, mixing and mobilising antimicrobial resistance?”, *Trends in Ecology and Evolution,* Vol. 26, No. 6 (2011)
[4] *Volkskrant*, “Weer dode in Duitsland door EHEC-bacterie; tientallen zieken”, 27-05-2011, http://www.volkskrant.nl/vk/nl/2672/Wetenschap-Gezondheid/article/detail/2438096/2011/05/27/Weer-dode-in-Duitsland-door-EHEC-bacterie-tientallen-zieken.dhtml (assesed 21-01-2012)
[5] H. Blaak et al., “Antibioticaresistente bacteriën in Nederlands oppervlaktewater in veeteeltrijk gebied”, Rijksinstituut voor Volksgezondheid en Milieu (RIVM), (2010)

the public health component to the most harmful bacteria for the civil community receiving their potable water from Oasen.

As this study aims to investigate fields that are different by character, the aquatic system and public health for example, it is impossible to provide answer by using one single discipline. These disciplines by themselves are inadequate to address this complex problem, because the disciplinary perspectives reveal only a portion of reality. Only by integrating these perspectives a broad and comprehensive understanding of the problem can be achieved.[6] Hence, interdisciplinary research is justified because the question is too complex to be solved adequately by one discipline and at least two disciplines give insights in the problem. In addition, the problem is an unresolved societal issue becoming more and more relevant.[7]

There are several potential disciplines that will contribute to the solution of this problem. Ideally, for solving this problem, knowledge is combined from the following domains:

- Aquatic systems → knowledge can be provided by hydrology (earth science)/environmental sciences

- Microbiology → knowledge can be provided by medicine/biomedical sciences

If new policy is required, one more domain should be added: governance. Applying new policy is a highly delicate procedure due to the many elements of society involved (e.g. stakeholders, environmentalists, recreation). The result is a wide range of disciplines that providing insights in this part of the problem:

- Governance→ knowledge can be provided by philosophy/political sciences/economics/communication

The aim of this thesis, however, is not to provide a new policy regarding water treatment, but to discuss the possible weaknesses in the current approach. A recent study by the World Health Organisation suggests that every risk assessment approach to groundwater protection should incorporate the three-stage combination of source, pathway and receptor. [8] These three stages represent the entire route from wastewater until drinking water. The source is the surface water in which ARB are present. The pathway is the route that the

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[6] Allen F. Repko, *Interdisciplinairy Research* (Sage Publications, Inc., 2008) 27-48
[7] Ibidem, 151
[8] World Health Organization (WHO), Protecting Groundwater for Health (*TJ International* (Ltd), Padstow, Cornwall, 2006), 82

water follows. This starts by the surface water infiltrating in the groundwater and ends at the water abstraction point of Oasen. The receptor is the water treatment company Oasen.

In the first chapter -the source- we will introduce the basic concepts of antimicrobial resistance and how bacteria acquire it. We will evaluate which bacteria are particularly dangerous for human health and the specific risk for our case.

In the second chapter –the pathway- we will investigate how these bacteria behave in the aquatic system and what pathway they follow through the ground. We will discuss what the mechanisms of groundwater-flow are and whether they are applicable to bacteria transport.

In the third chapter -the receptor- we will analyse water treatment carried out by Oasen. We will assess if the treatment is sufficient to remove all risks for public health. After this, we will return to the bacteria as described in chapter one to illustrate clinical importance

Case introduction[9]

In this study we will analyze the water treatment as carried out by the water treatment plant Oasen. Oasen is located in the province of South-Holland and its water collection area covers in approximation *'Het Groene Hart'* ('the green heart') in the east of the province. Recently, the plant has received licences to stay in operation till 2014. These licences are given for quality and environment.[10] 33 municipalities receive their water form Oasen all located in *'Het Groene Hart'*.[11] It has a total of 4048 km of pipes and abstracts in one year an average of 45 million m^3 of groundwater.[12] There are eight pumping stations in which water is abstracted for drinking water purposes. The abstracted water is largely replenished by infiltration water from the Rhine River. In contrast, meteoric water has little influence on the water quality because the aquifer is overlain with a semi-confining clay layer. The Rhine River cuts through this layer down into aquifer, providing an easy way for water to infiltrate into the aquifer. Contamination that enters the Rhine River up gradient of the abstraction point influences the water quality. Once the contamination arrives in the water collection area, it reaches the water treatment plants on average within four years.

In order to protect their water supply, Oasen protects its water collection area at three different levels. Firstly, the area directly above the abstraction point is physically protected by a fence surrounding it. Oasen owns the areas and makes sure that there is no grazing of cattle or manuring by farmers. Second, the concern lobbies at municipalities and companies to stimulate activities, such as the creation of natural reserves. Other activities are discouraged, such as the construction of gas stations. When the government is planning decontamination projects, Oasen advises which projects should have priority. Finally, by influencing local and European legislation, Oasen tries to protect the quality of groundwater in its water collection area.

Treatment

The water that is collected from the water collection area by Oasen, undergoes treatment. The first purification takes place in the soil, by means of a process called river bank filtration. The water has to have a residence time of at least 60 days. After this, all the pathogen

[9] All information in the case introduction, except where otherwise specified, provided by an interview with Harrie Timmer, hydrogeologist at Oasen, 25-11-2011
[10] Oasen, "Over Oasen", http://www.oasen.nl/over-oasen/Paginas/Oasen-krijgt-hercertificering-voor-Kwaliteit-Arbo-en-Milieu-Artikel.aspx (accessed 21-01-2012)
[11] Oasen, "Direct regelen", http://www.oasen.nl/direct-regelen/Paginas/Steeds-meer-gemeenten-heffen-precariobelasting-op-waterleidingen-artikel.aspx (accessed 22-01-2012), Oasen, "Waterleidingen", http://www.oasen.nl/waterleidingen/Paginas/Brandkraancontrole-draagt-bij-aan-schoon-leidingnet-Artikel.aspx (accessed 22-01-2012)
[12] Oasen, "Over Oasen", http://www.oasen.nl/over-oasen/Paginas/Drinkwaterkengetallen-Artikel.aspx (accessed 21-01-2012)

bacteria are dead, according to Oasen. The water treated by Oasen has a residence time of six months up to twenty years. There are several phases of the cleaning process performed at Oasen's stations. By sand filtering, large particles are filtered out of the water. Activated carbon removes undesired substances from the water. Subsequenty, carry-over filtering is applied, which is a softening process that serves the removal of ions. Finally, UV-filtering makes sure that the bacteria cannot reproduce. After this treatment the water is checked for Faecal Indicator Organisms (FIOs) that indicate remaining contamination.

In this process, Oasen does not monitor its water supply on non-pathogen bacteria; hence there is no control on antimicrobial resistance or endospores. The biomass growing on the insides of the pipes is checked and when it crosses a threshold value, the pipes are flushed. Oasen studies antimicrobial resistant bacteria in their water supplies.

Chapter 1 - Source

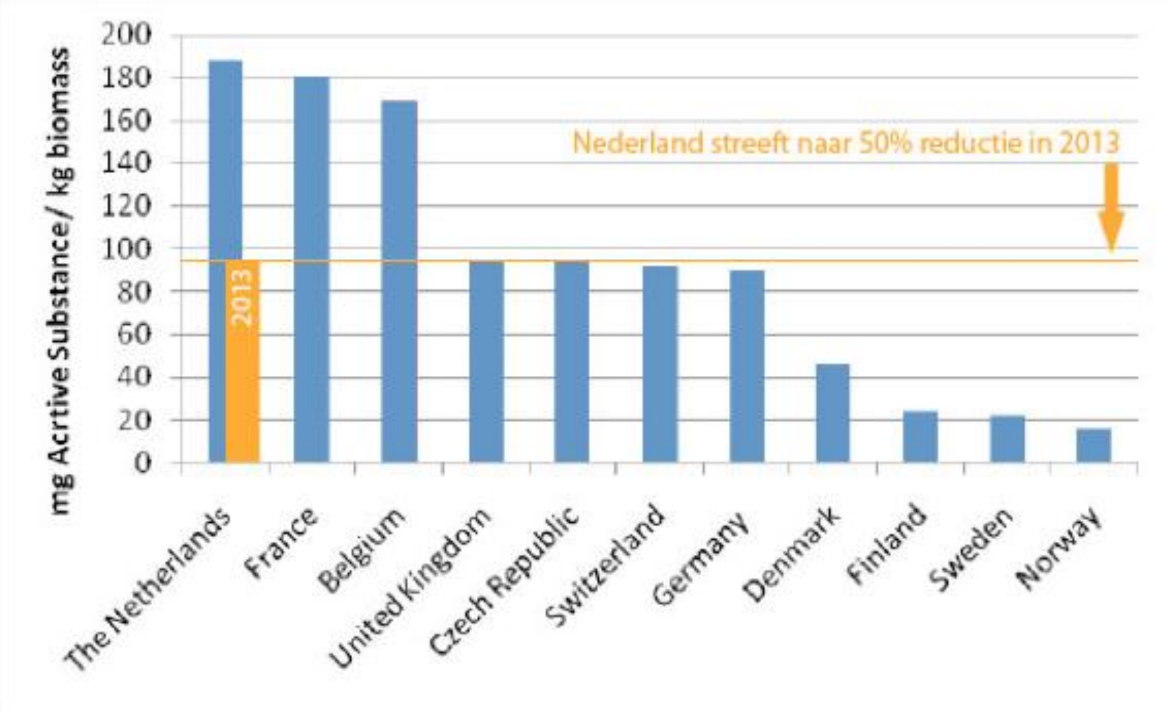
The discovery of penicillin in 1929 by Fleming is considered as one of the most important discoveries in modern times. Prior to this discovery, complicated diseases or even death could occur from what would seem, today, harmless infections. Penicillin became established as a wonder drug. Over the past several years many more types of antibiotics were introduced. Because of the broad applicability, millions of metric tons of antibiotics have been used all over the world. Unfortunately, this colossal demand for antibiotics has its downsides as well. Antibiotics cause selection pressure on microbes, leading to resistant bacteria entering the environment including the aquatic system. The presence of bacteria in the aquatic system is particularly worrisome since the aquatic system is the source of our potable water. Antimicrobial resistant bacteria (ARB) cause infections that are extremely challenging and sometimes even untreatable. Medical pundits are warning for the return of the pre-antibiotic era: a time in which bacterial infections will be life threatening again.

This chapter will focus on the source of potable water and the influence that high levels of antibiotic usage have on it. The sources of potable water are groundwater and surface water. First we will take a closer look on the annual usage of antibiotics (AB) and discuss its effects on the environment. After this, we will focus on resistant bacteria that are most dangerous for human health. The most important provokers of dangerous infections are gram-negative bacteria. Their most important mechanism of resistance is the production of extended spectrum β-lactamases (ESBLs). This is an enzyme that is capable of breaking down certain types of AB. To illustrate the impact of resistant bacteria we will use ESBL-producing bacteria as an example. ESBL-producing bacteria are an impressive example, because of their ability to develop new antibiotic resistance mechanisms in the face of the introduction of new antimicrobial agents, third-generation cephalosporins.[13] Finally, we will deal with the risks that play a role in our specific case. In the water collection area of Oasen dairy farms and a hospital are present. These companies use large amounts of AB, in particular β-lactam AB, resulting in the occurrence of antibiotic resistant bacteria (ARB) in surface water.

[13] D.L. Paterson and R.A. Bonomo, 'Extended-Spectrum β-Lactamases: a Clinical Update', in*: Clin Microbiol Rev.* 2005 18, 657-86

1. The annual usage of antibiotics in The Netherlands

In comparison with other European countries the antibiotic usage in livestock in The Netherlands until 2007 has been the highest of all European countries. The next Figure illustrates the differences between European countries.



[14]

Figure 1 comparison of mg active substance/kg biomass with ten other European countries

In 2008 there has been a decrease of more than 5% in AB usage, resulting in a 7th place compared to the other European countries. This 7th place is also caused by increased usage of other countries. However, the situation remains worrisome. ARB have a selective advantage to survive, resulting in an alarming proportion of resistant bacteria in some food-producing animals.[15] Figure 2, below, shows the sale of different types of AB allocated to livestock in the Netherlands.

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[14] Grave et al, 'Comparing of the sales of the veterinary antibacterial agents in ten European Countries', in: *J Antimicrob Chemother* 2010 9, 2037-402010

[15] Maran, 'Monitoring antimicrobial resistance and antibiotic usage in animals in the Netherlands in 2008', 2010, p 7-8

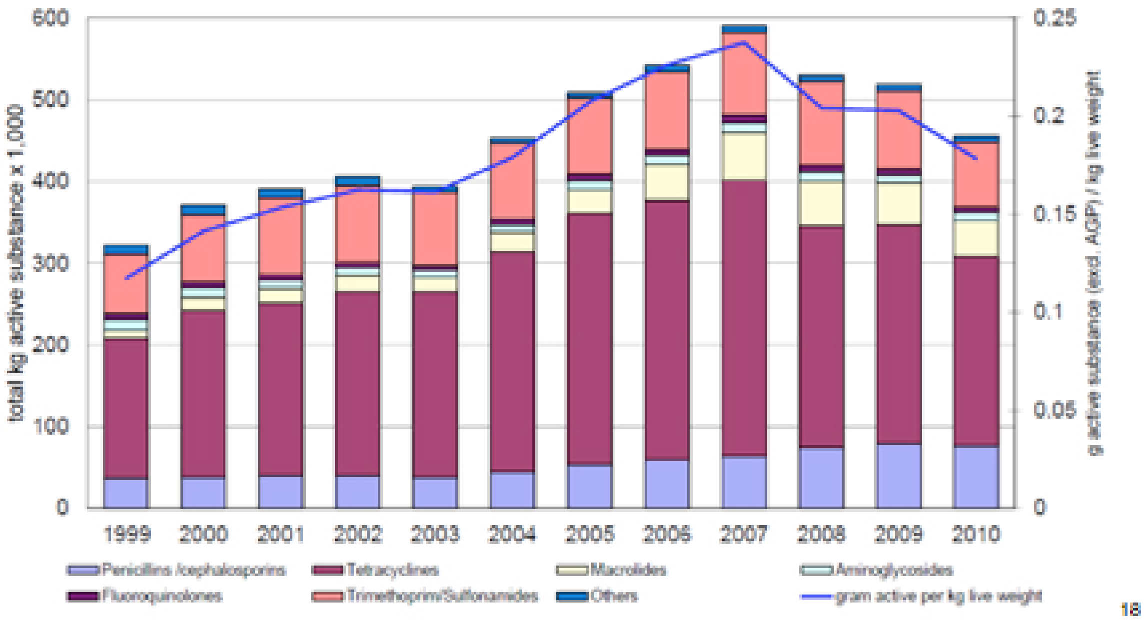

Figure 2 therapeutic Antibiotic sales 1999-2010

Indubitable, these ARBs influence the environment. Samples of surface water show a large percentage of resistant bacteria. According to the Dutch institute for public health and environment, *Rijksinstituut voor Volksgezondheid en Milieu* (RIVM), resistance levels were: 29% of *C. coli*, 36% of *E. coli*, 47% of *E. faecalis*, 80% of *E. faecium*, and 75% of the *staphyloccen* were resistant to at least one antimicrobal agent, whereas most of them were resistant to two or more antibiotics. Some isolates were even resistant to seven different antibiotics. The expected sources of the resistant bacteria are cattle farms, but there is also evidence that human sources, for example hospitals, are causing the contamination.[16]

2. Most threatening bacteria for human health

Since the 1940s there has been a rapid development of a wide range of antibiotics. Bacteria have proved to be adaptive to different antibiotics. Resistance is caused by mutations that have occurred over the past years. Mutations are changes in the DNA sequences that can occur spontaneously or can be caused by radiation or chemicals. Mutations that offer protection to antimicrobial agents offer a selective advantage to survive. Therefore the

[16] H. Blaak et al, 'Antibioticaresistente bacteriën in Nederlands oppervlaktewater in veeteeltrijk gebied', 2010*, RIVM rapport 70371903,* 9-10

percentage of resistant bacteria is increasing, while new therapies are long in coming.[17] When resistant bacteria cause infections, the risk exists of an untreatable infection.

Gram-negative bacteria of the *Enterobacteriaceae* family have often caused severe infections, such as urinary tract infections (UTIs), bloodstream infections, hospital- and healthcare-associated pneumonias, and various intra-abdominal infections.[18] Therefore, serious problems occur when these particular bacteria develop resistance to antibiotics. These mutations are often related to the production of β-lactamases by the bacteria.[19] This is a mechanism for breaking down β-lactam antibiotics, such as penicillins, cefalosporins and monobactams. They are often used in clinics, but have become less effective over the years, because of the resistance the bacteria have developed.[20]

Much research has been done in order to create new antibiotics against β-lactamase-mediated bacteria. The introduction of third-generation cephalosporins into clinical practice in the early 1980s was a major breakthrough. The cephalosporins had been developed in response to the increased prevalence of β-lactamases in, for example, E. coli and Klebsiella pneumoniae, that are potentially pathogenic bacteria. Third generation cephalosporins were effective against most β-lactamase-producing bacteria and they had the added advantage of lessened nephrotoxic effects compared to other ABs.[21]

Unfortunately, merely three years after the introduction of third-generation cephalosporins into clinical practice the first report of β-lactamases capable of hydrolyzing the third-generation cephalosporins was published. These new β-lactamases are called extended spectrum β-lactamases (ESBLs). There is no doubt that ESBLs will become increasingly complex and diverse in the future and improve their ability to break down β-lactam antibiotics.[22]

β-lactam antibiotics contain a β-lactam ring and inhibit cell wall synthesis by binding to membrane proteins, that are responsible for the final step in cell wall synthesis. These proteins are called penicillin-binding protein. If the cell wall cannot develop sufficiently, the

[17] Mims et al., 'Medical Microbiology', Mosby Ltd., 2007, 475
[18] D.L. Paterson, 'Resistance in gram-negative bacteria: Enterobacteriaceae' in: *AJIC: American Journal of Infection Control*, 2006 34, 20-28
[19] D.L. Paterson, Resistance in gram-negative bacteria', 20-28
[20] Reuland et al., 'ESBL in de kliniek: achtergrond, relevantie en epidemiologie', in: *Tijdschr Infect,* 2011 6, 127
[21] D.L. Paterson and R.A. Bonomo, 'Extended-Spectrum β-Lactamases', 657-686
[22] Ibidem, 657-686

cell will activate its autolytic system, causing cell lysis resulting cell death.[23] The image below gives a simplified explanation of the β-lactam function.

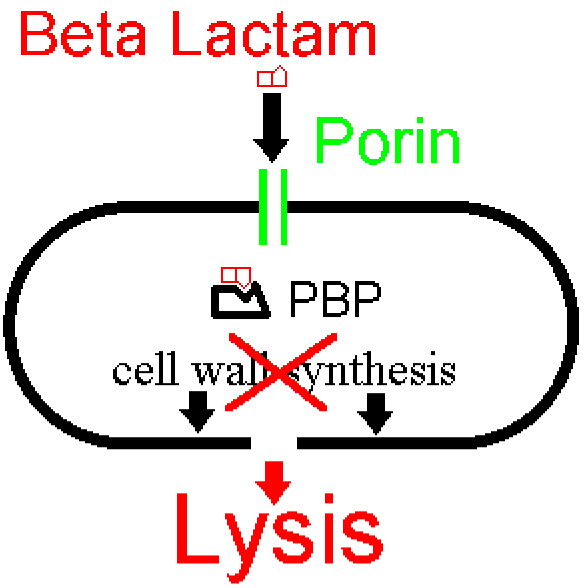

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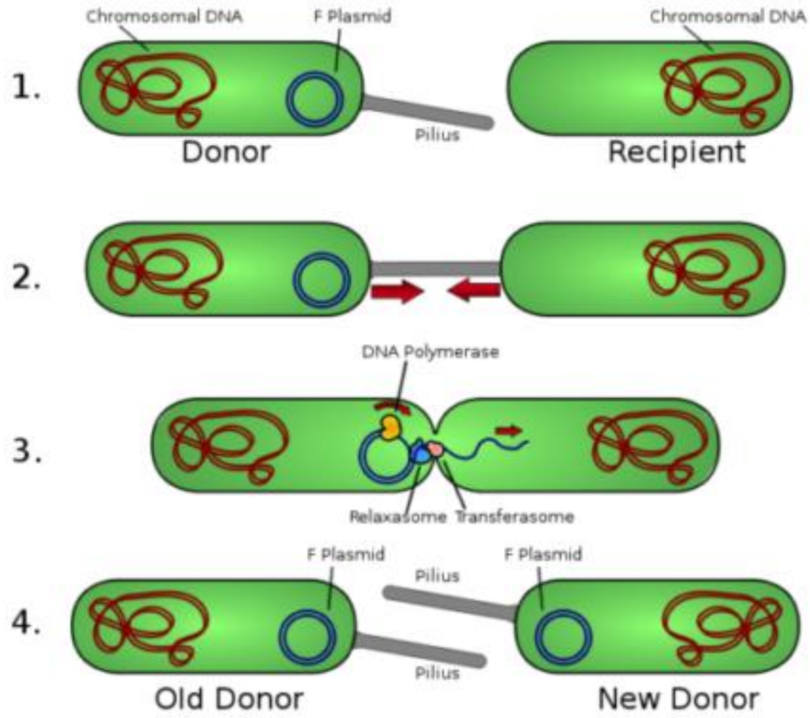
Figure 3 simplified image of β-lactam function

β-lactamases are enzymes that catalyze the degradation of the β-lactam ring. β-lactamase is encoded by genes that are found on chromosomes and plasmids.

Many different β-lactamase enzymes and ESBL enzymes have been found.[25] Numerous types of ESBL are known. Some are plasmidmediated. Plasmids are double-stranded and often circular DNA molecules that are independent of chromosomal DNA. These genes are considered to be 'mobile' as they allow horizontal transfer of genes to other bacteria. Plasmids are found in the cytoplasm of a bacterial cell and can be transferred to other bacterial cells, demonstrated in a simplified image below.

[23] Mims et al., 'Medical Microbiology', 478
[24] Lara Hopley and Jo van Schalkwyk. http://www.anaesthetist.com/icu/infect/Findex.htm#resist.htm (accessed 15-01-2012)
[25] Mims et al., 'Medical Microbiology', 479



[26]

Figure 4 conjugation in bacteria

Other ESBL-genes are located on chromosomal DNA. These genes can be transferred during reproduction. It is likely that all plasmid mediated ESBLs have chromosomal origins.[27]

It is obvious that resistant bacteria are relevant in clinical practice. When a patient is infected with resistant bacteria like ESBL-producing bacteria, empirically determined treatment may not be sufficient. This will possibly result in enhanced morbidity and in some cases mortality. As soon as culture results of the bacterium are available and an ESBL infection is confirmed, alternative therapies will be required. In most cases, these therapies are more toxic or less effective. Alternative therapies are often expensive and can sometimes only be administered intravenously.[28]

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[26] Blog Grrrlscientist, 'Food poisoning reminds us that bacteria do have sex', http://www.guardian.co.uk/science/punctuated-equilibrium/2011/may/30/microbiology-agriculture?CMP=twt_fd (accesses 17-02-2012)

[27] D.M. Livermore, 'beta-Lactamases in Laboratory and Clinical Resistance', in: *Clin Microbiol Rev.* 1995 (4), 557-84

[28] A. Reuland et al., 'ESBL in de kliniek: achtergrond, relevantie en epidemiologie', 130

3. Specific risks in our case

The water-collection area of Oasen is located in an environment where dairy farms and a hospital are located. A recent study of a Dutch working party VANTURES that monitors resistance en AB usage in animals, reveals that about 80% of the dairy farms in the Netherlands use β-lactam antibiotics, or a combination of antibiotics that contains β-lactams.[29] Since these antimicrobial agents work against bacteria that are dangerous for humans, the development of resistance is a threat to public health. β-lactam antibiotics are of particular importance because of the possible induction of ESBL-producing bacteria. The large-scale usage of AB in dairy farms has its effects on the water-collection area of Oasen. ARBs often end up in surface water. Often, this happens by manure of animals treated with AB. The manure can run from the land into surface water. Another possibility is the discharge of untreated or partially treated water into surface water. This can happen when hospitals, where people are treated with antibiotics, discharge their water.[30]

In conclusion we can state that heavy usage of antimicrobials leads to high levels of ARB, of which ESBL-producing bacteria are of particular importance. An infection by ARB can cause untreatable diseases, leading to morbidity and even mortality. ARB are commonly found in surface water. Chapter 2 will demonstrate the transportation and processes that take place in the soil. This will explain the condition and levels of bacteria that enter the well of Oasen. Chapter 3 will evaluate the water treatment carried out by Oasen.

[29] Maran, 'Monitoring antimicrobial resistance and antibiotic usage in animals in the Netherlands in 2008', 30
[30] H. Blaak et al, 'Antibioticaresistente bacteriën in Nederlands oppervlaktewater in veeteeltrijk gebied', 11-13

Chapter 2 - Pathway

In the previous chapter we have argued that high levels of antibiotic usage can lead to ARB entering the environment. The main focus of this chapter is to discuss whether or not ARB can enter the aquatic system and reach the water treatment plant in Gouda.

In our search to answer the question whether resistant bacteria in the aquatic system are a danger to public health by contaminating the potable water supply, we need to identify the source of the water, the pathway it takes before entering the water collection area of Oasen and whether the water is capable of transporting the ARBs from the source to the receptor.

In order to do this, we need to define the term 'groundwater'. Then we can take a closer look at the pathway of this water from the point of entering the system in recharge areas to the point of leaving the system at the discharge area (i.e. the water treatment plant in Gouda).

For the non-hydrologist the word groundwater refers to all the water residing under the surface. Within Hydrology this is called subsurface water, which is divided into more specific units based on the characteristics of the water body. (see Fig. 5) The main division in the subsurface water is between water in the unsaturated zone (vadose water) and in the saturated zone (groundwater) The unsaturated or vadose zone holds water along with air within the pores. In contrast, the pores in the saturated zone exclusively hold water. The water table or freatic surface is the surface that marks the division between the two zones. In this thesis the hydrological definition of groundwater will be used.

1. Porosity, permeability and hydraulic conductivity

The water treatment plant of Oasen in Gouda is located in the province of Zuid-Holland in the western part of the Netherlands. Its water collection area covers by approximation '*Het Groene Hart'* ('the green hart') of the Netherlands.[31] As can be concluded, groundwater abstraction is not restricted to the site of the plant, but takes up a large area in its surroundings. Because infiltration and transport of bacteria and pharmaceuticals is closely related to the subsurface[32], some physical characteristics of soil underlying this area should be known and understood. In the Netherlands the characteristics that are of importance are the (effective) porosity, permeability and hydraulic conductivity.[33]

[31] Harrie Timmer, interview, 25-11-2011
[32] Shannon Bartelt-Hunt et al. "Occurence of Steroid hormones and antibiotics in shallow groundwater impacted by livestock waste control facilities" *Journal of Contaminant Hydrology*, no. 123 (2011) 94-103
[33] F.C. Dufour, *Grondwater in Nederland,* (Rotterdam: Van de Rhee, 1998)

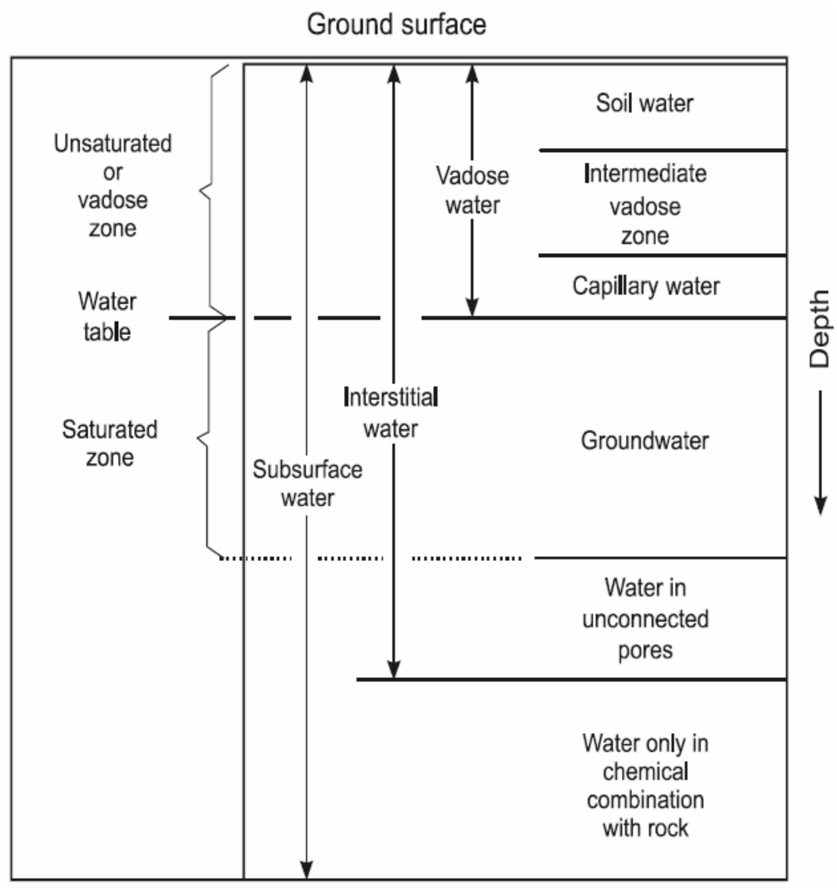

Figure 5 Classification of subsurface water[34]

The porosity is the ratio of the volume of pores/voids/cracks to the total volume of the body of rock, expressed as a decimal fraction or percentage.[35] Groundwater resides in these pores/voids/cracks and hence this ratio defines the maximum volume of water that can potentially be stored in the body of rock. The effective porosity is the ratio of the volume of pores/voids/cracks available for fluid transport to the total volume of the body of rock.[36] In other words, the effective porosity is defined as the proportion of the total volume that consists of interconnected pores able to transmit fluids.

The permeability is the degree to which a material is able to transmit fluids.[37] These characteristics define whether or not the soil layer can be considered as an aquifer or as an aquitard. An aquifer is a layer that has the potential to be a source useful for abstraction of groundwater. Characteristics favoured for aquifers are high porosity in combination with high

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[34] World Health Organization (WHO), Protecting Groundwater for Health (TJ International (Ltd), Padstow, Cornwall, UK, 2006), 24
[35] Tim Davie*, Fundamentals of Hydrology*, (New York: Routledge, 2008), 58
[36] Eddy Moors et al. *Hydrologische woordenlijst*, (Delft: Nederlandse Hydrologische Verenging, 2002), 86
[37] Tim Davie, Fundamentals of Hydrology, 58

permeability, i.e. capable of storing a large amount of water and transmitting it with ease. An aquitard is a layer that scarcely allows water to pass or only with difficulty. The most important characteristic of an aquitard is low permeability. This means that an aquitard can have the capability to store a large amount of water, but disables abstraction of it.

The hydraulic conductivity takes into account the permeability, gravity and the viscosity and the density of the fluid.[38] The hydraulic conductivity defines the rate of movement of a fluid through a porous medium. It is defined as the flux per cross-sectional area of a porous medium under the influence of a unit hydraulic gradient.[39] The hydraulic gradient will be explained in more detail in the next paragraph.

Case

Porosity and permeability are characteristics of the sediment, therefore knowledge of the material making the sediment is crucial. De Vries (2007) argues that "[t]he deposits participating in the present-day hydrological cycle consist predominantly of Plio-Pleistocene, medium to coarse, fluvial sands with a thickness that increases north-westward to more than 300m. […] The lower part of the aquifer partly consists of a succession of coarse and fine, marine sediments of the Early Pleistocene Maassluis Formation and the Pliocene Oosterhout Formation […] The Plio-Pleistocene aquifer is the most important source for public water supply in the east and south"[40] (see Fig. 6).

For our casus the situation far left in Figure 2 is the situation to deal with. As can be seen in Figure 3, the layer at the surface is an aquitard/confining layer, this means that there is little to no exchange of water. De Vries (2007) formulates it as follows: "The clay and peat-containing soils in this area [coastal lowlands] are strongly anisotropic due to low-permeable horizontal sediment layers. This obstructs vertical penetration of the precipitation surplus and stimulates concentrated lateral drainage through the upper meters." This statement is in accordance with the information Harrie Timmer gave us in the interview. He told us that there was almost no infiltrating water from the surface. Figure 4 shows that there is indeed a large difference in hydraulic conductivity of the clays and the medium to coarse fluvial sands. The hydraulic conductivity of clay is very low and the hydraulic conductivity of medium to coarse sand is in between moderate and high.

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[38] Stephen Marshak, Earth, Portrait of a Planet, (New York: W.W.Norton & Company, 2007) G-10
[39] Australian Government, "Hydraulic Conductivity Measurement" http://www.connectedwater.gov.au/framework/hydrometric_k.php (accessed 21-01-2012)
[40] J.J. de Vries, Geology of the Netherlands, Ed. Th.E. Wong, D.A.J. Batjes & J. de Jager (Royal Netherlands Academy of Arts and Sciences, 2007), 295–315

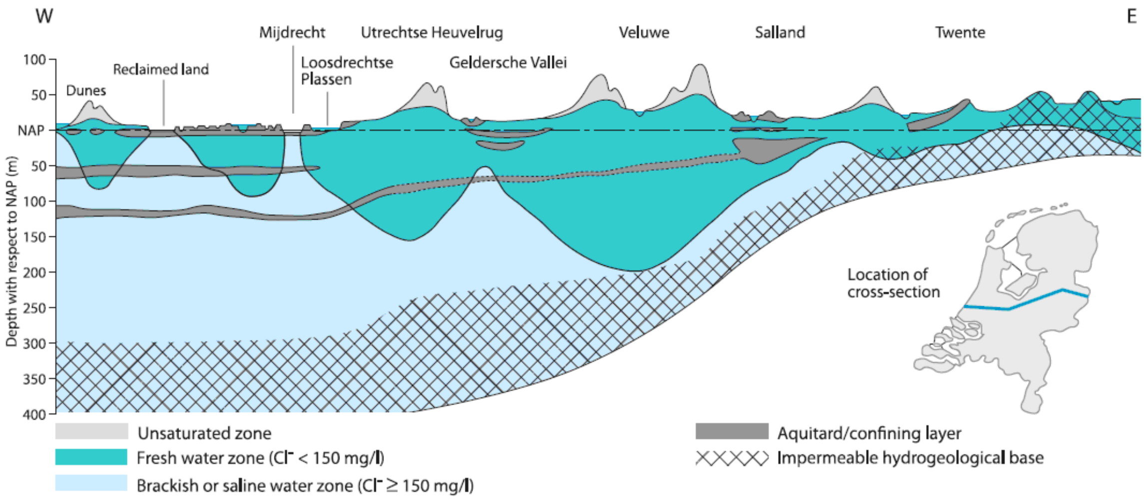

Figure 6 Schematic topographic-hydrogeologic east-west section showing the approximate depth of the fresh-brackish water interface (brackish > 150 mg Cl/l); length of section ca. 200 km[41]

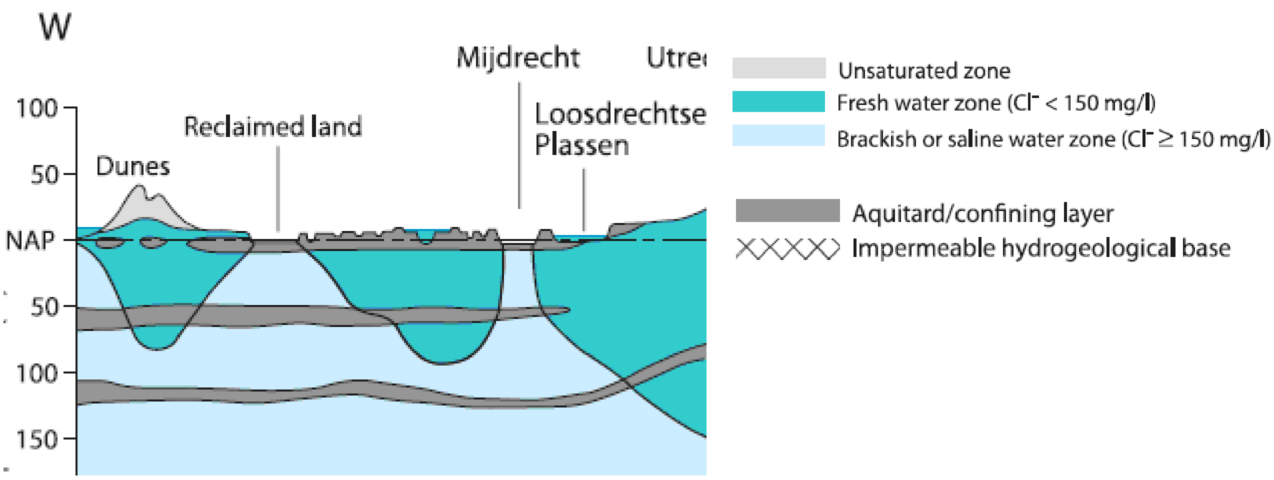

Figure 7 Magnification of figure 2

[41] J.J. de Vries, Geology of the Netherlands, 302

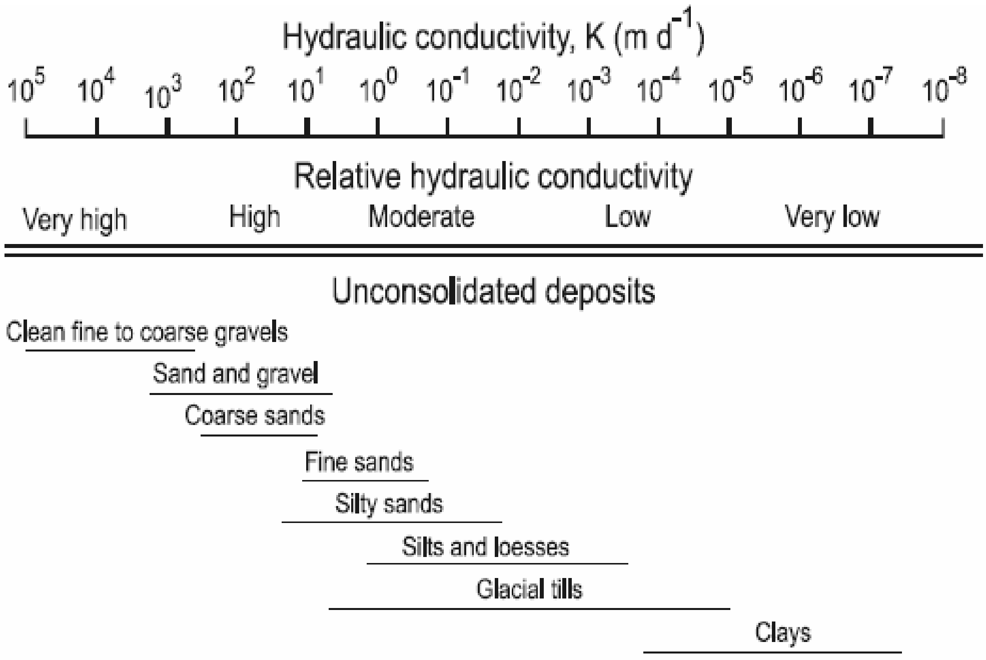

Figure 8 Relative hydraulic conductivity related to different sediments[42]

2. Fluid flow

At the water treatment plant in Gouda groundwater is abstracted. For the source to remain useful, this abstracted water needs replenishment. If there is, as mentioned above, no infiltrating meteoric water, what feeds the replenishment?

In order to abstract water one must assume that fluid flow is possible in the subsurface. The subsurface consist of alternating aquifers and aquitards, restricting groundwater flow to specific layers. Besides this division in behaviour of groundwater, the earlier mentioned division between the unsaturated and the saturated zone comes into play as well.

Forces driving fluid flow

Water movement in the unsaturated zone is solely due to gravitational forces; water movement in the saturated zone is a more complex process involving gravitational forces as well as concentration/pressure differences. These differences can cause water to move side- and upwards.[43]

In the saturated zone, the flow of groundwater through an aquifer is governed by Darcy's Law, which states that the rate of flow is directly proportional to the hydraulic

[42] World Health Organization (WHO), Protecting Groundwater for Health, 32
[43] Marshak, *Earth,* 667

gradient. The hydraulic gradient consists of two components, the elevation head and the pressure head. The elevation head is the difference in height between two locations due to geographic conditions causing a potential energy that can drive groundwater flow. The pressure head is the difference in pressure at the locations storing another energy that can drive the groundwater flow.[44]

The pressure at a location is caused by the weight of the water column at this point. This implies that the deeper one descends in the groundwater, the higher the pressures. The pressure of the overlying rock column does not contribute to the pressure used here, because the grain contacts counterbalance this. The pressures can differ from location to location. Groundwater tends to flow in the direction of the lowest pressure.[45]

The total amount of potential energy inside the saturated zone is given by the gravitational force and the internal pressures; this energy is called the hydraulic head. Groundwater will flow from locations with high hydraulic head to locations with low hydraulic head. The difference between two hydraulic heads along the flow path is called a hydraulic gradient.[46]

Groundwater flow velocity

Flow velocities of groundwater are in the order of 4 - 500 meters a year. This is extremely slow compared to surface water velocities. The difference between these two can be explained by looking at the physical properties the flow of groundwater has to overcome. First of all, groundwater can not flow in straight lines. It is bounded by the small passages and conduits contained in the material. It may be clear that the number of conduits as well as the size of the conduits and the straightness of the conduits influence the speed at which a fluid can travel through the material. In addition, due to the very small conduits the friction and electrostatic affinity between the fluid and the walls of the conduits is significant.

As mentioned before, the flow of groundwater through an aquifer is governed by Darcy's Law. The mathematical expression of this law is:

$$Q=K(\Delta h/j)A \qquad \text{(Eqn. 1)}$$

In which **Q** stands for the discharge, the volume of water passing through a defined area in a certain amount of time.

[44] Marshak, *Earth,* 669
[45] Ibidem, 669
[46] Ibidem, 669

K stands for the hydraulic conductivity; which takes into account the permeability, gravity and the viscosity and the density of the fluid. The hydraulic conductivity defines the rate of movement of a fluid through a porous medium. It is defined as the flux per cross-sectional area.

A stands for the area of the porous medium through which the fluid flows, measured perpendicular to the flow direction.

Δh/j stands for the hydraulic gradient; which is the change in hydraulic head along the flow path.[47]

In general, Darcy's Law states that

1) Fluid flows faster through permeable material than through impermeable material
2) Fluid flows faster when the differences in hydraulic head are larger.

However, the actual mean groundwater pore (linear) velocity of groundwater (v) differs from the Darcy velocity (q) that can be abstracted from this general law as flow can only occur through the effective porosity (n_e) of the formation. This velocity may be quantified by modifying the Darcy equation.[48]

The Darcy equation:

$Q = K(\Delta h/j)A$ (Eqn. 2)

For calculating the Darcy velocity (q) the equation can be rewritten to

$q = Q/A = K(\Delta h/j)$ (Eqn. 3)

As the hydraulic gradient is often referred to as i, the equation can be rewritten to

$q = Ki$ (Eqn. 4)

The Darcy equation is conventionally written with a minus sign because flow is in the direction of decreasing hydraulic heads.[49]

$q = -Ki$ (Eqn. 5)

When calculating the mean groundwater pore (linear) velocity of groundwater (v), by taking into account the effective porosity (n_e), the equation becomes

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[47] Marshak, *Earth,* 669
[48] World Health Organization (WHO), Protecting Groundwater for Health, 87
[49] Ibidem, 31

$v = -Ki/n_e$ (Eqn. 6)

In conclusion, before one can calculate the velocity at which groundwater flow, one needs to know the hydraulic conductivity, the hydraulic gradient en the effective porosity.

Case

The river cuts through the aquitard into the Plio-Pleistocene aquifer[50] and is thereby able to have a direct access to the groundwater supply. Due to overpressure of the river in comparison with the substrate the water is eager to leave the river and infiltrate into the soil. The hydraulic conductivity in medium to coarse fluvial sands is approximately 10^1 m d^{-1}. (see Fig. 8) The effective porosity can be established by experiments.

If these three variables are known, one can calculate the average groundwater flow velocity in the aquifer and then it is possible to estimate the resident time of the water in the ground.

3. Groundwater transport mechanisms

Groundwater contains all kinds of solutes (e.g. calcium, iron) and small particles (e.g. grains of sand) that can be transported along with the moving groundwater. Above we discussed what causes the flowing, but we did not touch upon the mechanisms facilitating the transport of dissolved matter. This dissolved matter is transported by three mechanisms: advection, dispersion and diffusion. These mechanisms are discussed in more detail below.

Advection is the transport of dissolved solute mass present in groundwater due to the bulk flow (movement) of that groundwater. Advection alone (with no dispersion or reactive processes occurring) causes a non-reactive solute to advect (move) at the mean groundwater pore velocity. All solutes undergo advection, however, reactive solutes are subject to influences by other processes detailed below.[51] The mean advective velocity of non-reactive solutes is equal to v in equation 6 and is normally estimated by knowledge of the hydrogeological parameters of this equation.

Mechanical dispersion causes spreading of solute and hence dilution of concentrations, it arises from the tortuosity of the pore channels in a granular aquifer and of the fractures in a consolidated aquifer and the different speeds of groundwater within flow channels of varying width. It affects all solutes.[52] Dispersion causes dissolved-phase plumes

[50] Harry Timmer, interview, 25-11-2011
[51] World Health Organization (WHO), Protecting Groundwater for Health, 86

[52] Ibidem, 86

to broaden both along and perpendicular to the groundwater flow direction (see Fig. 9). In addition dispersion causes mixing of the dissolved-solute plume with uncontaminated water and hence concentration dilution as well as plume spreading.[53] Mechanical dispersion can arise at the pore scale due to

1) Fluids moving faster at pore centres due to less friction
2) Larger pores allowing faster fluid movement
3) Routes of varying tortuosity around grains[54]

Transverse horizontal spreading arises from flowpath tortuosity and molecular diffusion due to concentration gradients. Transverse vertical spreading occurs for similar reasons, but is generally more restricted due to predominantly near-horizontal layering of geologic strata. Overall, a hydrodynamic dispersion coefficient D is defined for each direction (longitudinal, transverse horizontal, transverse vertical).[55]

$$D = \alpha v + D^* \qquad \text{(Eqn. 2)}$$

In which **D** is the hydrodynamic dispersion coefficient

α is the geologic media dispersivity

v is the actual mean groundwater pore (linear) velocity of groundwater (see eqn. 6)

D* is the solute's effective diffusion coefficient

Dispersion parameters are most reliably obtained from tracer tests or, less reliably, at the larger (>250 m) scale, by model fitting to existing plumes. Collated values have yielded simple empirical relationships to estimate dispersion, e.g. the longitudinal dispersivity is often approximated to be 0.1 (10 per cent) of the mean plume travel distance (Gelhar, 1986). However, such relationships are very approximate.[56]

Are the groundwater transport mechanisms applicable to bacteria transport?

In our search to map the pathway of pathogen contamination in the aquatic system we have considered the transport mechanisms for groundwater and its solute components. At this point we can take a closer look at the transport of pathogens within the subsurface.

[53] World Health Organization (WHO), Protecting Groundwater for Health, 88
[54] Ibidem, 88
[55] Ibidem, 89
[56] Ibidem, 89

As stated above hydrologists make a distinction between vadose water in the unsaturated zone and groundwater in the saturated zone. In order to answer the question how pathogens are transported in the aquatic system it is important to take this distinction into account because the behavior of the pathogens in these two zones is different.

In order for the contamination to reach the receptors it has to move away from its source. This can occur through the natural pathways or by human intervention. Human can create so called rapid transport pathways (RTPs) which can directly connect the source and the receptor. The creation of these RTPs cannot explain all groundwater source contamination and it is now widely accepted that the transport of microbial pathogens within groundwater systems is a important mechanism for waterborne disease transmission.[57]

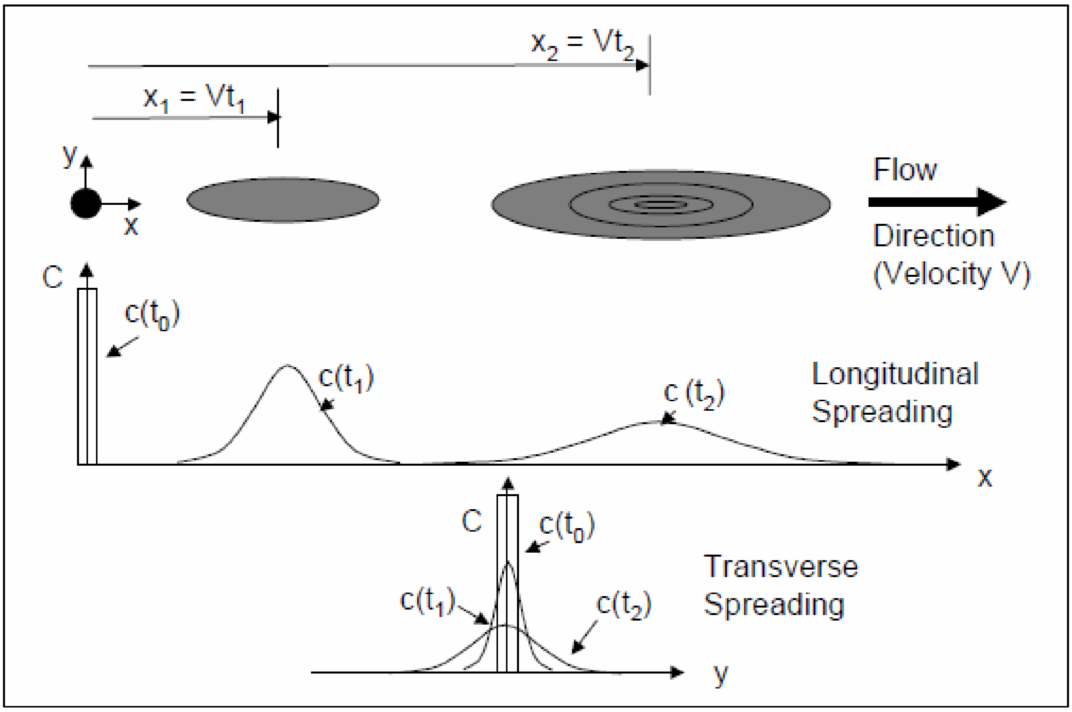

Figure 9 Dispersion of a pulse release of dissolved-solute plume[58]

Unsaturated zone

Hydrogeological processes in the unsaturated zone are often complex en hence the behavior of pathogens is not easy to predict. However, research has revealed that the attenuation of pathogens is the most effective in the uppermost soil layers. The presence of protozoa and other predatory organisms, the rapid changes in soil moisture and temperature, competition

[57] World Health Organization (WHO), Protecting Groundwater for Health, 61
[58] Ibidem, 88

form the established microbial community and the effect of sunlight at the surface combine to reduce the level of pathogens in this zone.[59]

Moisture

The moisture content is of great importance for the transport of pathogens from the surface into the subsurface. Even in relatively dry periods the soil contains enough moisture to facilitate the downward movement of pathogens into the subsurface. Due to the thin film of moisture the pathogens are brought in close contact with the surface of the particles thus enhancing the probability of adsorption of the pathogens onto this surface. Adsorption may retard the downward motion of the pathogens, but it seems to protect the organisms as well. It has been shown to reduce the inactivation of both viruses and bacteria, thereby increasing the persistence of pathogens beyond what is predicted form the inactivation rates in groundwater.[60] The inactivation is observed as the inability to replicate and form a visible colony.[61] During events of high rainfall the pathogens can be remobilized into the water and be transported rapidly downwards with the water percolating down into the subsurface.[62]

Size

The size of the pathogens controls, to some extent, their ability to move. Pores in soils and rocks are known to vary in size, thereby excluding all pathogens that are larger then the pore size from moving through the porous medium. This process is called physical filtration or straining. However, the protective effect of straining can be bypassed by migration through preferential pathways or human intervention (e.g. RTPs caused by sewers).[63]

Saturated zone

As well as in the unsaturated zone, the bacteria that make it down in to the saturated zone are subject to the same processes of attenuation. Thus processes natural die-off, adsorption onto particles, filtration/straining, predation and dilution are still active, but under the conditions of natural or artificially induced flow. Microorganisms in groundwater are transported by the same mechanisms as solute particles.[64] This results in a migration and spreading of the pathogens in space and time, which as a result can lead to contamination of increasingly large aquifer volumes as the pathogens move downgradient.

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[59] World Health Organization (WHO), Protecting Groundwater for Health, 61
[60] Ibidem, 66
[61] Knudson et al, “Inactivation of biological threat agents with nonionizing radiation”, *Radiation Inactivation of Bioterrorist Agents*, 2005, 169
[62] World Health Organization (WHO), Protecting Groundwater for Health, 62
[63] Ibidem, 64
[64] Ibidem, 65

Characteristics of the microorganism	Aquifer/soil (environment) properties
Size	Groundwater flow velocity
Shape	Dispersion
Density	Pore size (intergranular or fracture)
Inactivation rate (die-off)	Kinematic/effective porosity
(Ir)reversible adsorption	Organic carbon content (solid)
Physical filtration	Temperature
	Chemical properties of groundwater (pH, etc.)
	Mineral composition of aquifer/soil material
	Predatory microflora (bacteria, fungi, algae, etc.)
	Moisture content
	Pressure

Table 1 Factors effecting transport and attenuation of microorganisms in groundwater[65]

Depending on the type of aquifer the radius of the migration from the source of contamination is several tens of meters up to a few hundred meters.

Case

The aquatic system at the site of Oasen consists of two major components: a peaty aquitard and a sandy aquifer. As presented the bacteria transport is influenced by five factors, i.e. natural die-off, adsorption at particles, filtration/straining, predation and dilution. We will discuss for each of these factors.

The uppermost layers of the soil (10m) consist of clayey en peaty layers of a Holocene age. Figure 8 shows that this has a very low hydraulic conductivity and will not transmit water very easily. As mentioned this layer acts as a semi-confining aquitard, restricting water percolating from the surface into the subsurface, and favours horizontal transport and hence horizontal dilution. Table 1 and Figure 6 tell us that silts and clays have small pore diameters and that there is a class of bacteria that will experience physical filtration due to this. Clays have a high potential that binds pollutants to their surface, in this way retarding the motion.

The aquifer below this semi-confining layer consists of Plio-Pleistocene, medium to coarse, fluvial sands. Figure 8 shows that this has a high hydraulic conductivity and allows water to flow easily through the material. In addition, Figure 10 shows that the pore diameter in sands is large enough for almost all bacteria to pass, so there will be no physical filtration

[65] World Health Organization (WHO), Protecting Groundwater for Health, 65

on behave of this. Due to these characteristics dilution can take place in both horizontal and vertical direction.

Class	Microorganism	Size
Virus	Bacteriophage	0.02-0.2 µm diameter
	Poliovirus	0.03 µm diameter
Bacteria	Bacterial spores (*Bacillus*, clostridia)	1 µm
	E. coli	0.5 µm x 1.0 µm x 2.0 µm
	Salmonella typhi	0.6 µm x 0.7 µm x 2.5 µm
	Shigella spp.	0.4 µm x 0.6 µm x 2.5 µm
Protozoa	*Cryptosporidium* oocysts	4.0-6.0 µm diameter
	Giardia	7.0-14.0 µm diameter
	Enteroamoeba histolitica	20-25 µm diameter

Table 2 Size of different pathogens occurring in groundwater[66]

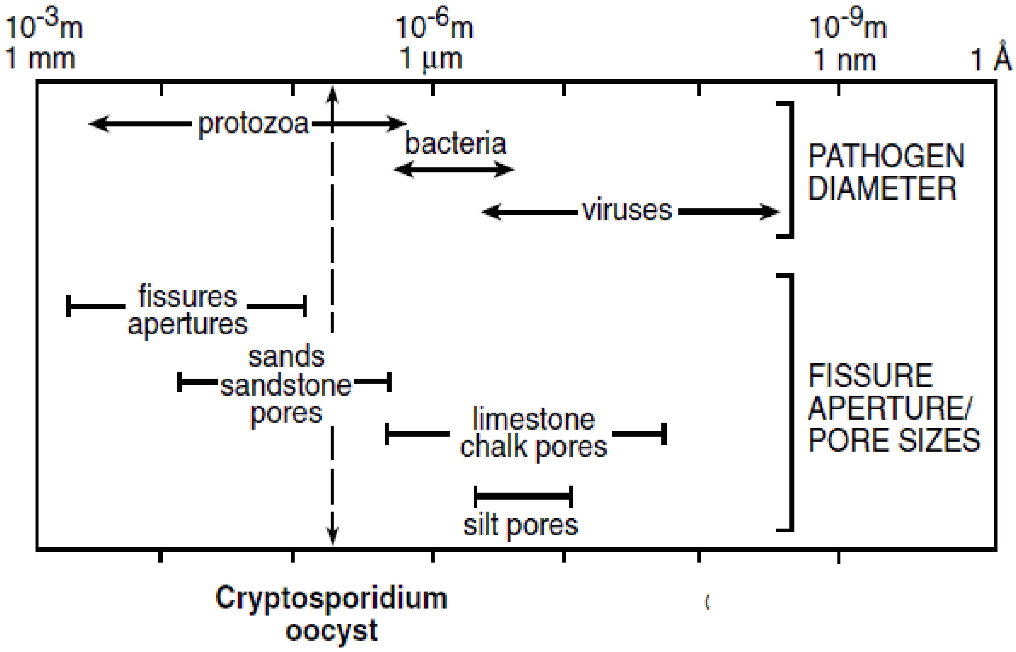


Figure 10 The size of different pathogens and the pore size of sediment[67]

Conclusion

According to the criteria above there is a possibility of bacteria infiltrating into the water collection area of Oasen. As mentioned above bacteria itself can travel several tens of meters up to a few hundred meters in the aquifer. Natural die-off, predation and dilution (no straining, due to the large diameter of the pores in sands) will attenuate the contamination cloud. Adsorption onto particles may retard the motion, but offers the bacteria a relatively safe environment, enabling them to persist for a longer period in the aquifer then would be

66 World Health Organization (WHO), Protecting Groundwater for Health, 63
67 Ibidem, 63

possible without this adsorption. Once adsorbed onto particles it is possible that during periods of high rainfall the bacteria suspend once more into the water, enabling the ARB to infiltrate further into the aquifer.

Because both pathogen and non-pathogen bacteria are subject to the processes of groundwater movement and there is no restricting physical filtering, there is reason to believe that ARB can reach the water-collection area in Gouda.

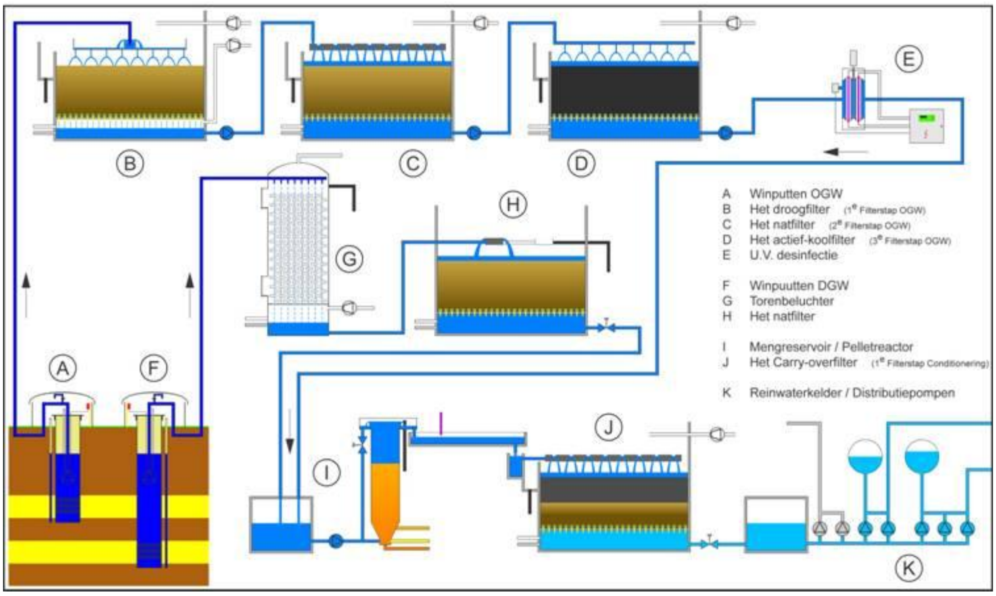
In the next chapter we will examine the receptor, i.e. the water treatment plant Oasen, Gouda.

Chapter 3 - Receptor

The previous chapter presented an analysis of the pathway that resistant bacteria follow during their journey through the soil. The conclusion that was drawn is that the presence of resistant bacteria in the water well of Oasen is convincingly probable. In this chapter we will discuss the receptor, which is the water treatment. First we will describe the water treatment carried out by Oasen. After this, we will evaluate each step in this process in order to examine the effectiveness of the water treatment. Finally, we will discuss the clinical importance of possible ARB in potable water. Therefore we will go back to the ESBL bacteria as described in chapter 1, in order to illustrate the impact of ARB.

1. Water treatment adopted by Oasen

Raw water available from River Bank Filtrate and ground water is not directly suitable for drinking purposes. Oasen uses different steps in their process, illustrated in the next Figure. This Figure shows that water from the superficial wells (A) undergoes a more intensive treatment than water from the deeper wells (F).



[68]

Figure 10 water treatment adopted by Oasen

The water from well A first undergoes Sand Filtration (B, C). This method is used to trap large particles out of the water. Secondly, activated carbon (D) is used to remove non-

[68] Powerpoint: Ground water abstraction by Oasen, E. Meuter et al.,Oasen, date unknown

settlable particles from the water that are too light to settle and are not large enough to be trapped during the process of filtration. Subsequently, ultra-violet (UV) treatment (E) is used for disinfection of the passing water. A light source emits UV-waves that can inactivate harmful microorganisms. The dosage used by Oasen is 20 mJ/cm^2. Thereafter the water enters a mixing tank (I) to mix source water that originates from different wells. Finally, the water is softened by a carry-over filter (J). The softening process is a technique that serves the removal of ions, in most cases magnesium and calcium, which are known to clog pipes and to complicate soap and detergent dissolving. After this treatment process the water enters the distribution systems.

Water from well F will first undergo aeration. This is a process to in raise oxygen levels in the water (G) before continuing to the Sand Filtration. Afterwards the water enters the mixing tank (I) and pursues the same schedule as well A.[69]

The safety of a water source is frequently verified by testing the presence of indicator microbes. These microbes are used as indicators for the presence of pathogens. The common term used for these organisms is: faecal indicator organisms (FIO). An ideal FIO is always present in faecal waste, easily detectable and more durable than any pathogen.[70] Oasen uses the following organisms as FIOs:

- *E. coli*
- total coliform bacteria
- *Campylobacter*
- Giardia lamblia (parasite)
- bacteriophage (virus indicator)
- enteroviruses

Summarizing, the measures taken against bacteria in potable water are the following: ground water use, UV-filter and the culture of FIOs.

2. Evaluation

In this part we will evaluate the effectiveness of the different measures against present ARB that are taken by Oasen. Measures taken against bacteria in potable water are: a. ground water use, b. the culture of FIOs and c. UV-radiation.

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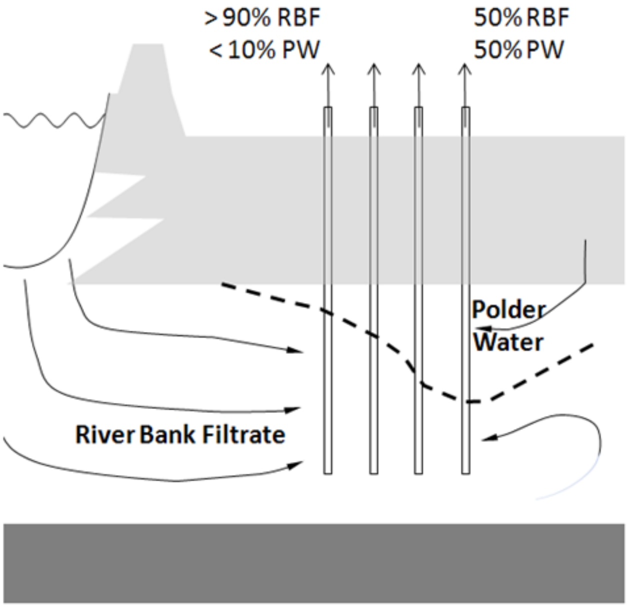
[69] Interview Harrie Timmer, 25-11-2011
[70] World Health Organization (WHO), Protecting Groundwater for Health, 52-54

a. Groundwater use

Chapter 2 explained that Oasen uses relatively young ground water partially originating from riverbank filtration and from meteoric water, infiltrated into the aquifer. The image below shows that the closer the wells are located to the river, the more water originates from riverbank filtrate.



[71]

Figure 11 The water that enter the wells

This picture does not reveal the different depths, where the wells are located. Water from deeper wells and distant from the river is considered to be microbially safe drinking water. Therefore, Oasen aims to lower bacterial contamination by using deep ground water from distant wells. Recall from chapter 2 that ground water is often shielded from the immediate influence of contamination by the overlying soil and unsaturated zones. In these zones, it is assumed that pathogenic microorganisms are attenuated by prevailing conditions. Therefore the risk of pathogens being transported into ground water and producing a threat to public health, was considered low over the past years.[72]

However, recent studies show that this assumption is inaccurate. Although the microbial contamination in ground water is likely to be lower than in surface water, recent data show that up to 70% of ground water sources is contaminated with one or more FIO. This entails probable faecal contamination containing reproductive bacteria. Nevertheless

[71] Powerpoint: Ground water abstraction by Oasen, E. Meuter et al.,Oasen
[72] World Health Organization (WHO), Protecting Groundwater for Health, 55, 56

many water treatment companies use ground water sources for public supply with a minimum level of treatment.[73] Oasen applies minimal treatment to the water that was won from the deeper wells, as we have seen in the previous part of this chapter (fig 10). The absence of UV-treatment might be of crucial importance as some (resistant) bacteria might survive in the deep ground water.

Another discovery within this context is the process of horizontal gene transfer in bacteria. The main sources of ARBs in soil near farms are the application of animal manure to agricultural soils and leakage from manure lagoons. Studies about the transport of faecal bacteria show that the survival times of faecal bacteria in soil are limited. However, horizontal gene transfer from these bacteria to indigenous soil bacteria might mediate persistence of resistance genes in soil. Manure carries high loads of resistant bacteria plasmids.[74] Recall from chapter one that plasmids are found in the cytoplasm of a bacterial cell and can be transferred to other bacterial cells. Plasmids can contain genes coding for antibiotic resistance. For example, intergenic horizontal transfer of resistant genes caused outbreaks of vancomycin-resistant *Staphylococcus aureus*. Transfer of plasmids can occur between distantly related species.[75]

We can state that although faecal microbials do not always survive in soil, resistant bacteria can be transferred to soil bacteria that do reach the well. If soil bacteria are non-pathogenic, genes can be passed to pathogenic bacteria in later stages of water treatment. Resistant genes might even be passed to pathogenic bacteria after the water treatment or even inside the human intestinal system. Further research should elucidate this process.

b. Culture of FIOs

A second measure that Oasen takes is the culture of FIOs. Oasen searches for the presence of FIOs in drinking water as an indicator of the presence of pathogens. Unfortunately there is no such thing as an ideal FIO, and one should be aware of its inadequacy. For one thing a difficulty is that although the density of FIOs provides a measure of probability of the presence of pathogens, studies (Schmoll et al. 2004) show that FIOs do not give an absolute certitude of the presence/absence of pathogens. The group of pathogens is diverse and

[73] World Health Organization (WHO), Protecting Groundwater for Health, 56

[74] H. Heuer et al., 'Antibiotic resistance gene spread due to manure application on agricultural fields.' In Curr Opin Microbiol. 2011 14, 236-43

[75] Heuer, 'Antibiotic resistance gene spread due to manure application on agricultural fields.', 236-43

differs highly in transport and attenuation of the population during transport. These characteristics cannot be wholly represented by only a small number of FIOs.[76]

A recent study aims to elucidate that although FIOs like E. coli*,* thermotolerant coliforms, and/or intestinal enterococci have been used as FIOs for many years, they have limitations. The number of *E. coli*, thermotolerant coliforms, and/or intestinal enterococci in faeces is relatively low and they sometimes grow in the environment as well.[77] This indicates that using FIOs can be not only insensitive but also unspecific.

Another obstacle is that some bacteria, even FIOs can become dormant in the environment. In this state bacteria are viably, but non-culturable. Therefore their presence cannot be verified in a culture. An important example of a FIO that can become dormant is *E. coli.* Its possible pathogenicity and resistance remains unaltered. Some bacteria can even survive on more extended and extreme conditions by forming endospores. Endospore formation occurs usually in gram-positive bacteria, some of which are pathogenic like *Clostridium* and *Bacillus*.[78] When conditions are more favorable the endospores can return to normal vegetative bacteria, which can divide to produce more cells.

In conclusion we can state that the culture of FIOs is far from an ideal examination for ARB. Even if FIOs are absent, the possibility of present pathogens cannot be ruled out. By culturing FIOs, Oasen searches for faecal contamination, but does not show considerations for resistant genes in environmental bacteria. Dormant bacteria and endospores will pass this measure taken by Oasen as well. After FIO culture, the presence of (pathogenic) ARB is not inconceivable and should therefore be taken seriously.

c. UV-radiation

Oasen uses an UV fluence of 20mJ/cm^2. UV light does not destroy or damage cellular structure. Nevertheless, UV-light prevents the cell from reproducing. According to Harrie Timmer, a hydrogeologist at Oasen, the dosage of 20 mJ/cm2 is sufficient even in most unfavorable conditions to inactivate bacteria. Recent studies cast doubt on the effectiveness of UV-radiation because of occurring resistance in bacteria. Like antibiotic drugs, UV-radiation is an antibiotic treatment. Due to accidental mutations bacteria can become resistant to UV-radiation, which will offer them a selective advantage to survive. Several studies have reported an increased UV resistance of environmental bacteria and bacterial

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[76] World Health Organization (WHO), Protecting Groundwater for Health, 55
[77] J.J. van der Wielen and G. Medema, 'Unsuitability of Quantitative Bacteroidales 16S rRNA Gene Assays for Discerning Fecal Contamination of Drinking Water' in: Appl Environ Microbiol. 2010 76 (14), 4876-4881
[78] World Health Organization (WHO), Protecting Groundwater for Health, 70

spores, compared to lab-grown strains. This means that higher UV fluences are required to obtain the same level of inactivation.[79]

Microbial inactivation can be expressed in Microbial Inactivation Credit (MIC). In a recent study (Hijnen et al. 2006), inactivation expressed in logarithmic units was used to quantify required UV fluence to inactivate bacteria. To calculate required UV fluence the following parameters are requisite: 1. type of microbial, 2. the concentration microbial in the influence, 3 maximum accepted concentration set up by the government. Table 3 shows the correlation between UV fluence and the microbial inactivation credit (MIC) for certain types of microbials and a set concentration of influence.

	Required fluence (mJ/cm^2)			
MIC required (log):	**1**	**2**	**3**	**4**
Bacillus subtilis[a]	56	111	167	222
Clostridium perfringens[a]	45	95	145	—[b]
Streptococcus faecalis[a]	9	16	23	30
Legionella pneumophila[d]	8	15	23	30
Shigella sonnei[d]	6	13	19	26
Salmonella typhi[a]	6	12	17	51
E. coli O157[d]	5	9	14	19
E. coli[a]	5	9	14	18
Campylobacter jejuni[d]	3	7	10	14
Yersinia enterocolitica[d]	3	7	10	13
Legionella pneumophila[d]	3	6	8	11

[79] W.A. Hijnen et al.,'Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water', in: *Water Res.* 2006 40(1), 3-22

	Required fluence (mJ/cm²)			
MIC required (log):	**1**	**2**	**3**	**4**
Shigella dysenteriae[d]	3	5	8	11
Vibrio cholerae[d]	2	4	7	9

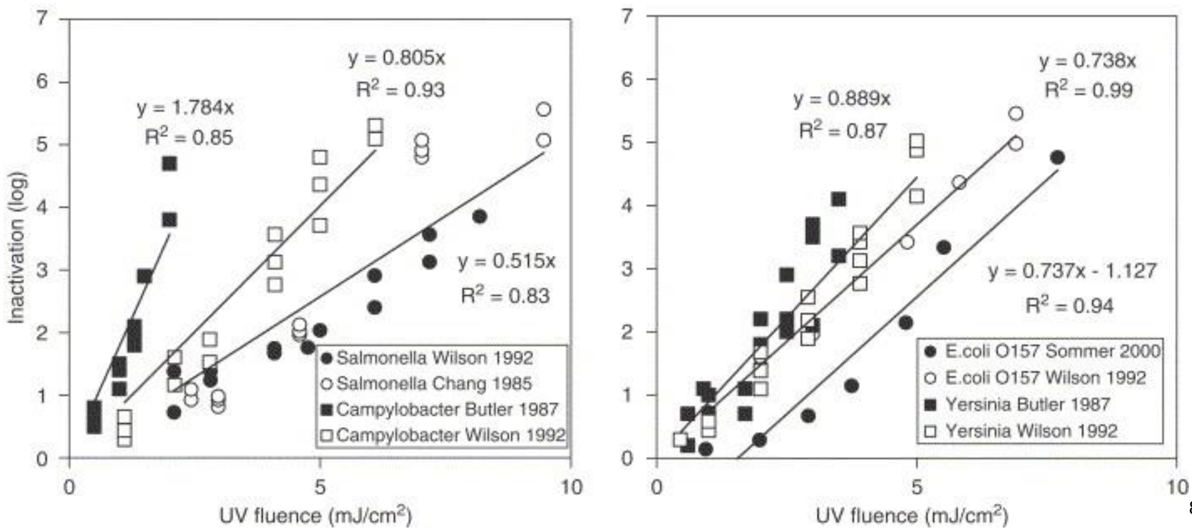
[a] Environmental spp.

[d] corrected for environmental spp.

[e] No value due to tailing. [80]

Table 3 Bacteria inactivation by UV-radiation

The next Figure illustrates the inactivation of gram-negative bacteria under set conditions. This is a graphic example that does not correspond to the situation in our case.



[81]

Figure 12 graphic illustration of bacteria inactivation

Different MICs are required for different bacteria. According to W.A. Hijnen government standards differ widely. For example: in drinking water the maximum concentration of a non-

[81] W.A. Hijnen et al.,'Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water', 3-22.

pathogen *E. coli* is 1/100 ml. If influence contains 100/100ml *E. coli* a MIC of 2 log is required.[82] When we have a look at Table 3 we find that a fluence of 9 mJ/cm2 is required (low pressure lamp UV254nm), so the standard 20 carried out by Oasen is sufficient in this case. Conversely, when we have a look at a pathogen *E. coli, E. coli* 0157, standards are stricter. 1.2e-6 per liter is the maximum set up by the government. Imagine the same influence of 100/100ml. In this case a MIC of 8.9 log is required, which is not even presented in table 3. This will come down to a fluence of 45 mJ/cm2 (low pressure lamp UV254nm).[83] In this case the standard of 20 mJ/cm2 will not suffice.

In our case the fluence is set at 20 mJ/cm2. Whether this level offers a safe condition is unclear, since not al parameters are at our disposal. We don't know the quantitative influence in our case, which makes it impossible to calculate required fluence. Therefore further research is requisite.

3. ESBL

The preceding evaluation has casted doubt on the reliability of water treatment carried out by Oasen. To put a meaning on the risks described before, we will go back to ESBL-producing bacteria in order to illustrate the clinical importance. Recall from chapter 1 that ESBLs are dangerous for human health since an infection with ESBL-producing bacteria can lead to complicated or even untreatable diseases. Most ESBL infections arise in hospitals or nursing homes. However in the last three years there have been several reports about community-acquired infections.[84]

In chapter 1 we described the AB use in the water collection area Oasen. About 80% of AB contains a β-lactam AB, which makes the occurrence of ESBL-producing bacteria in surface water likely. Chapter 2 presented the pathway of bacteria resulting in the conclusion of possible ESBL bacteria in water wells from Oasen. Whenever ESBLs enters water treatment of Oasen, the measures it faces are the following: ground water use, culture of FIOs and the UV-filter.

Ground water use is not fully reliable as 70% of the groundwater sources is contaminated with one or more FIO. This entails faecal contamination and the possibility of ESBL originating from manure. Besides that horizontal transfer fills a part. Since genes

[82] Email communication with W.A. Hijnen, author of 'Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: a review' 18-01-2012
[83] Ibidem
[84] D.L. Paterson and R.A. Bonomo, 'Extended-Spectrum β-Lactamases: a Clinical Update', 657-86

coding ESBL are often located on plasmids, the presence of ESBL-producing bacteria in the water is possible.[85]

FIO culture is not an ideal examination for ESBL. FIOs are unspecific and insensitive for the presence of ESBL-producing FIOs and only indicate a probability of faecal contamination. Environmental bacteria carrying ESBL genes are not examined. This is impractical since ESBL genes can be transferred to other bacteria. The presence of ESBL after a negative FIO culture is not inconceivable.

It is hard to tell whether the UV-filter is sufficient to inactivate all present bacteria in drinking water. Therefore in this stage it is impossible to draw conclusions on ESBL inactivation. Further research is required to determine ESBL influence and calculate MIC of ESBL.

Summarizing, although this study needs further deepening, the imponderables of water treatment carried out by Oasen have been evaluated and revealed. Furthermore the correlation between the sudden upsurge of community-acquired ESBL infections and possibly contaminated drinking water needs further exploration. Despite the many uncertainties we encounter, we can conclude that the risk of ARB in drinking water cannot be ruled out and that a correlation between drinking water contamination and community-acquired infections with ESBL is not excluded.

[85] Reuland et al., 'ESBL in de kliniek: achtergrond, relevantie en epidemiologie', 127

Integration

So far we have presented a multidisciplinary approach to the problem, in which the knowledge from both hydrology and medicine are juxtaposed. While both disciplines aim to construct a better understanding of the natural phenomena we observe, it is interesting to notice that for a long time scientists in both disciplines tried to achieve their goals in isolation. Considering the present day challenges in the field of environmental bacteria resistance and human health, it is absolutely necessary for scientists in both disciplines to cooperate and integrate their latest findings.

It is interesting to notice that the combination of medicine and hydrology is a fruitful one. Their fields of research generally do not overlap. However, both disciplines provide background information that is necessary to answer questions that rise in the other discipline. An example of this that appeals to one's imagination is provided by the history of the life sciences. When the British naturalist Charles Darwin was struggling to formulate a coherent theory to explain the origin of species, he did not succeed until he heard of the findings of the Scottish geologist Charles Lyel. In his Principles of Geology (1830 – 1833), Lyel stated, based on newly discovered earth layers, that the earth was millions of years older than scientists and theologians previously thought. This gave Darwin the framework he needed for his theory of evolution by natural selection. After all, a younger earth would not provide nature with enough time to account for the large amount of species by the process Darwin was proposing.[86] This example shows that research into geology and research into the life sciences is often interlinked. As in Darwin's and Lyel's research, our research involves complementary insights that resulted from interlinking two disciplines: hydrology and medicine. Each single discipline would be necessary, but not sufficient to answer the questions formulated in this case study and therefore it is absolutely necessary to adopt an interdisciplinary approach.

In this chapter we will identify differences, similarities and compatibilities between the disciplinary insights in order to discover a common ground. Finally we will answer our main question by integrating these insights.

Both disciplines belong to the natural sciences, which implies that their research field is nature itself. Assumptions in both disciplines overlap to a certain extent. Basic assumptions often used in natural sciences are:

[86] C. Buskes, *Evolutionair denken; de invloed van Darwin op ons wereldbeeld* (Amsterdam: Uitgeverij Nieuwezijds, 2006), chapter 1.

- Nature is orderly
- We can know nature
- All phenomena have natural causes
- Nothing is self-evident
- Knowledge is based on experience
- Knowledge is superior to ignorance[87]

It is interesting to notice that both our disciplines do not fully comply with this list and there are some discrepancies to distinguish.

Medicine is a discipline that assumes one truth that we can investigate. All phenomena do have natural causes and nothing is self-evident. We can investigate the truth through experimental research. In medicine it is not generally assumed that nature is orderly. Unlike physics, biology or medical sciences do not always follow a law of nature. Although potential medication, for example, is developed using laws of nature, for safe clinical use this does not suffice. Lab tests, animal experiments and clinical trials are always required. This example shows that natural laws are part of medicine but are never sufficient to discover the truth. Laws of nature often cannot capture biological phenomena like the human body. Medical sciences, like biology, therefore often use statistics or experimental data upon which theories are based.

In this study we analyzed antibiotic resistance and bacteria mutations. In medicine, adaptations in genome sequence of bacteria are generally considered as randomly occurring phenomena. Modifications due to environmental changes are unique and do not suggest the presence of a pre-existing program that regulates mutations.[88] The point at issue is that besides the natural laws that are commonly used in medicine, randomness occupies a major place in processes of the individual human body.

Hydrology, like medicine, assumes one truth that we can investigate. All phenomena do have natural causes and nothing is self-evident. However, one always works with an approximation of the truth, not the truth itself. Theories used in the Earth Sciences are not considered to be absolutely true, but they describe natural phenomena and should be in concordance with observations and knowledge available. Models used in the Earth Sciences should be capable of giving an explanation that is consistent with observations. When

[87] Frankfort-Nachmias & Nachmias in A. Repko, interdisciplinary research, *SAGE Publications Inc* 2008, 89
[88] G.N. Amzallag, ‘Adaptive changes in bacteria: a consequence of nonlinear transitions in chromosome topology?’ in: J Theor Biol. 2003 7;229 (3):361-9

multiple theories are available and valid Ockham's Rasor is often used to indicate the best explanation.

It is customary to verify theoretical laws through experimental research. Unlike in medicine, hydrology rather searches for laws of nature than experimental outcomes. However, experimental outcome can alter the perception of the theoretical law. Within the Earth Sciences models containing natural laws often underlie experiments. If such experiments yield other outcomes then predicted by these models, then the parameters involved in the model are regarded to be false, but not the natural law underlying it. This is due to the fact that a model is considered to be a simplified reflection of reality, because nature as a whole is to complex to be described in a model.

In this study we used a model for the estimation of the groundwater flow velocity. Parameters in this model are often established by fieldwork and experiments. As said, when experimental outcomes and predicted outcomes are inconsistent, the parameters involved in the model are regarded to be false, but not the natural law underlying it. Hence, in contrast to medicine, no randomness occurs.

In Hydrology, just as in Medicine, theory, models and experimental data are strongly interlinked. However, in Hydrology, in contrast to Medicine, natural laws are always considered to be correct, all 'randomness' can be explained by incorrect or unknown parameters.

These differences in assumptions lead to different concepts of truth. In medicine the outcomes of experimental research is assumed to be true, even if it is based on statistics and not on laws. Exceptions and randomness in medicine are common and conclusions based on statistics are the most workable conclusions available. In hydrology truth can only be approached using models that contain natural laws. If experimental research yields inconsistencies, laws are not considered to be wrong, but rather incomplete.

When we integrate insights originating from both disciplines, one should be aware of different assumptions and therefore different epistemological basic principles. The value of conclusions that rise from medical experimental studies should not be considered as absolute truths, rather as the most workable hypotheses of the present day. Therefore, using medical insights in our case, our final conclusion will not be an absolute truth, rather a workable hypothesis.

While keeping the latter conclusion at the back of our minds, we can integrate our findings of this research. The insights provided by hydrology and medicine complement each other well and do not contradict. Therefore the insights of one discipline can easily be added to the insights of the other discipline. The following table lists the insights derived from both disciplines.

Insight	**Discipline**
In the Netherlands the annual use of antimicrobials is substantially higher than in other European countries.	Medicine
High levels of antimicrobials cause resistance in bacteria, which is dangerous for public health.	Medicine
Resistant bacteria enter surface water.	Medicine/environmental sciences
There is an interaction between surface water and groundwater	Hydrology
Groundwater is our main potable water resource	Hydrology
Transport of ARBs is limited by groundwater flow- and/or sediment characteristics.	Hydrology.
Due to groundwater abstraction at Oasen, an artificial concentration gradient is created, forcing river water to infiltrate in the aquifer.	Hydrology
The aquifer and/or groundwater flow in this case study does not limit the transport of ARBs.	Hydrology
ARBs can reach the water treatment plant in Gouda	Hydrology
Water treatment may not be sufficient to eliminate all ARB’s in potable water	Medicine

Table 4 a linear sequence of insights

This table shows that our main question can be answered using a linear sequence of insights originating from both disciplines; hence both disciplines are necessary to reach the final insight that eventually leads to the answer of our main question. The insights that each single discipline generates in this case are insufficient or even fragmented. Creation of a common is not necessary, but a mere organisation of the fragments will suffice and give rise to a shared understanding. The connection between the insights will be established along the line of source, pathway and receptor. This integrative framework will provide a more comprehensive understanding of the problem, just by linking the insights as shown in table 4.

A final conclusion follows from the organisation of insights. This conclusion will not be an absolute truth, rather a workable hypothesis.

Conclusion

In this study we analyzed to what extent ARB could be present in potable water originating from water treatment company Oasen and the dangers for public health. We concluded that high dosages of antimicrobials leads to high levels ARB, of which ESBL bacteria are of particular importance. Infections caused by ARB are often complicated to treat and sometimes even untreatable. This will lead to co-morbidity or even mortality. Resistant bacteria are commonly found in surface water. There is a possibility of these bacteria infiltrating into the aquifer. Bacteria can travel several tens of meters up to a few hundred meters in the aquifer. Natural die-off, predation and dilution (no straining, due to the large diameter of the pores in sands) will attenuate the contamination cloud. Adsorption onto particles may retard the motion, but offers the bacteria a relatively safe surrounding; enabling them to persist for a longer period in the aquifer then would be possible without this adsorption. Once adsorbed onto particles it is possible that during periods of high rainfall the bacteria resorb into the water, enabling the ARB to infiltrate further into the aquifer. Because bacteria both pathogen and non-pathogen are subject to the processes of groundwater movement and because of the absence of restricting physical filtering, there is reason to believe that resistant bacteria can reach the water treatment plant in Gouda. Measures taken by Oasen against bacteria in their water are the following: ground water use, the culture of FIOs and UV-radiation. Although further investigation is required it is likely that the use of groundwater and the culture of FIOs are not sufficient to eliminate all ARB in the water. Whether the UV-radiation will inactivate the leftover ARB below national standards is unclear. Despite the uncertainties we encounter, we can conclude that the risk of ARB in drinking water cannot be ruled out and that a correlation between drinking water contamination and community-acquired infections with ESBL is not excluded.

Discussion

In this study many subjects with a possible relevance have not been investigated thoroughly enough, due to a lack of time and available means. Therefore further research is required on the following subjects.

Firstly, due to the use of models in order to estimate groundwater flow velocities and motion we encounter uncertainties. Models present a simplified perception of reality and contain parameters that can be incorrect. General values are known for the parameters involved in Darcy’s Law and calculating the dispersion rate. However conditions can vary within one single aquifer and general values can deviate from the actual values. Further investigation should be done in defining and refining these parameters. The parameters can be defined by experiments with tracers in the specific area of Oasen.

Secondly, we found that horizontal gene transfer can be responsible for the transfer of genes coding for resistance in the soil. We think that genes coding for resistance may even be passed to pathogenic bacteria after the water treatment or even inside the human intestinal system. Further research should elucidate if and if so to what extent this process occurs. Horizontal gene transfer in ESBL bacteria deserves a special focus.

In addition, we presented that research has already revealed that endospores are nonculturable. Further research is required to analyze whether ARB form endospores as well, in particular ESBL. If this is the case, ESBL cannot be trapped by FIOs or inactivated by UV-radiation. The consequences of the presence of ESBL endospores in groundwater would be serious, since this would imply the probable presence of ESBL in drinking water.

Subsequently, further research is desired on the parameters that are required for the calculation of the microbial inactivation credit. In our case the fluence of UV-radiation was set at 20 mJ/cm2. Whether this level offers a safe condition is unclear, since we don’t know the quantitative influence in our case.

Finally, more research is necessary in order to explore a possible correlation between ESBL bacteria in drinking water and the sudden upsurge of community acquired infections.

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