Slide 1: Title and introduction to presentation

The title of my presentation today is "The presence of amoeba-resistant bacteria in water distribution systems". This is a topic that has not yet been addressed in South Africa but has been receiving a lot of attention internationally in recent years.

Slide 2: List of contents

In order to understand amoeba resistant bacteria and their risk to the health of humans, I will provide background information about free living amoeba, the so-called 'viable but non-culturable stage' often seen in bacteria in water distribution systems, the importance of biofilms. I will then give a short introduction to the study the NIOH has recently started to study the presence of amoeba resistant bacteria in water distribution systems and their possible risks to workers in industries where they are found.

Slide 3: Background of free living amoebae (FLA)

The interest in free-living amoebae (FLA) has grown substantially during the last decades as a result of the demonstration of their pathogenicity and their role as reservoirs for Legionella bacteria.

FLA are present world wide and have been isolated from natural sources including soil, water and aerosols where they feed on bacteria, fungi and algae. As such they play a very useful role in controlling bacterial populations, increasing soil fertility and cycling nutrients in aquatic food chains.

FLA are also present in most man-made water distribution systems and been isolated from drinking water, cooling towers, swimming pools, hydrotherapy baths, HVAC systems. As inhabitants of these systems, they have the potential to cause human infections.

Slide 4: Stages of development of FLA

FLAs have at least two stages of development:

<u>Vegetative (feeding) form (trophozoite)</u> – this is an active, feeding form. They feed by a process called phagocytosis.

<u>Resting form (cyst)</u> – Cysts are formed when food requirements are not met and when environmental conditions are not favourable. They excyst when environmental conditions become favourable again. The cyst generally has two layers called the ectocyst and the endocyst. Some species also have a third layer, the mesocyst. The cyst walls are very hardy and can withstand harsh conditions for long periods of time.

Some amoebae, such as Naegleria species, have a third stage of development, the <u>flagellate stage.</u>

The structure of the cysts has certain characteristics that make it possible to distinguish different genera of FLA microscopically.

Slide 5: Stages of development continued

When conditions are favourable the amoebae are present in their active form but during conditions of stress, cysts are formed for survival. These cysts are very hardy and can survive for long periods of time, often harbour live bacteria and can 'excyst' when conditions become favourable again. This cycle continues as conditions change.

Slide 6: Pictures of amoebae in active and cyst form

A, B and C in the picture represent active, feeding amoebae (trophozoites). In picture D the cysts of *Acanthamoebae* can be seen.

Cysts of different genera of FLA have different forms and characteristics. These cyst forms are distinquishable by microscopy and are used to differentiate among the different amoebae.

Slide 7: Pathogenicity

There are 2 pathogenic FLA of importance in the South African setting: several species of the genus *Acanthamoeba* and one species of the genus *Naegleria* (*Naegleria fowlerii*) .

ACANTHAMOEBA SPECIES

Several of the Acanthamoeba species are implicated in human disease. Trophozoites enter the body through the respiratory tract, ulcerated or broken skin and invade the central nervous system. Cysts have also been demonstrated in tissue samples. Acanthamoeba species cause dermatitis, granulomatous amoebic encephalitis, pneumonitis and keratitis.

Slide 8: Pathogenicity continued

NAEGLERIA FOWLERII

N. fowlerii enters the human body through the nose, and infects the brain and spinal cord where it causes primary amoebic meningo-encephalitis. If treatment is not started soon after infection, the disease is fatal within 7-10 days of infection.

Slide 9: Amoeba resistant bacteria (ARB)

A number of microorganisms including bacteria, fungi and viruses, have become resistant to FLAs and are able to survive, grow and exit FLAs after internalization, The FLA thus act as hosts for these microorganisms. These organisms can exist in water distribution systems as free-living (planktonic), attached to surfaces (sessile) or intracellular form.

Although these organisms may be bacteria, viruses or fungi, this presentation deals with amoeba resistant bacteria (ARB) only.

Several of these ARBs are fastidious, intracellular bacteria and are regarded as human pathogens. Some have been known for many years and others are considered as newly emerging or re-emerging pathogens. Between 1995 and 2001 **11** bacterial species were recognised as forming part of the ARBs. By 2004 there were **46** ARB described. The total currently stands at **163** species belonging to 82 genera. The table illustrates that nearly 50% of these ARB have now been confirmed to be pathogenic to humans, while 5 species have recently been shown to be potentially pathogenic. Work on the rest of the species is currently being done to establish whether they are pathogenic to humans.

Slide 10: Table

I have listed a few of the best known pathogens that have recently been added to the list of ARB. So far, 8 of the Legionella species and 30 species of Mycobacterium have been added to the list. Other very important members of the ARB are Vibrio cholera (which causes cholera), Salmonella, Shigella and Helicobacter (which cause gastric symptoms – diarrhoea, peptic ulcers).

Slide 11: Table

This table lists the symptoms and diseases of the most common bacteria that have been listed as ARB recently, with their corresponding Hazard Group as listed in the Hazardous Biological Agents Regulations of 2001.

Slide 12: ARB pathogenicity illustration

In the illustration above I have taken Legionella as an example, because it has been used as a model for studies of the pathogenicity of ARB.

In water distribution systems the amoebae feed on bacteria. A number of these bacteria are digested as food, but the majority resist digestion and multiply within the host. This multiplication continues until the amoeba ruptures and sets free thousands of live bacteria. This cycle then continues, with more amoeba feeding on these bacteria, some being digested and the others again multiplying to be spread when the amoeba ruptures.

In many cases (for example during water treatment) the amoebae, with the live bacteria inside, may encyst until the conditions are favourable again for them to excyst. The process of multiplication of bacteria and rupture of the amoeba then continues as before.

Slide 13: Infectivity in human cells

This process is very similar to what happens with intracellular bacteria within the human body. The blue drawing on this slide represents a human cell, where the same process takes place. The bacteria are taken up by a process called 'phagocytosis' but instead of being destroyed by the cell, the bacteria multiply within the cell until they are set free in high numbers. This is typical of many intracellular bacteria, for example Legionella.

Slide 14: Important ARB

The ARB listed on this slide are considered to be of importance in the South African context (with special reference to occupational health setting). They are all established pathogens. The dates when they were first recognised as ARBs are given in brackets.

Legionella species are well known for their role in legionellosis (Legionnaires' disease and a less serious flu-like disease called Pontiac fever).

Legionella-like amoebal pathogens (LLAP) were first discovered in the early 1990s. They are very closely related to legionella species but cannot be cultured under laboratory conditions without amoebae, hence their title. Molecular studies have made it possible to include a few of these bacteria in the genus *Legionella* recently.

Chlamydia species, more specifically *C. pneumoniae* was added to the list of ARBs in 1997 and shortly afterwards, also in 1997, another bacterium closely related to C. pneumoniae known as *Parachlamydia acanthamoebae (Hall's coccus)* was added to the list. *C. pneumoniae* is known to cause respiratory infections and has been implicated in atherosclerosis and Alzheimer's disease. *Hall's coccus* may cause respiratory tract infections, bronchitis, aspiration pneumonia and community acquired pneumonia especially in AIDS patients. There have been reports of *Hall's coccus* being implicated in Kawasaki's disease.

Members of the *Mycobacterium avium complex (MAC)* were added to the list in 1998. *MAC* are often isolated from AIDS patients with respiratory disease but other infections have been reported. *Vibrio cholera* and *methycillin-resistant Staphylococcus aureus (MRSA)* need no introduction.

Slide 15: Viable but non-culturable bacteria

When exposed to environmental stress most bacteria enter a "viable but non-culturable" (VBNC) state, characterised by slow metabolism and respiration and temporary loss of the ability to be cultured under laboratory conditions. While in the VBNC state the bacteria decrease in size and undergo several changes which may give rise to false negative results when testing water samples for bacterial contamination. However it is important to remember that these bacteria are alive and will return to their normal active state when conditions are favourable. Also important is that these bacteria are just as pathogenic as their normal, active counterparts. Legionella species are well known for the long periods of time they can exist in the VBNC state especially after treatment of water with biocides.

Bacterial ecologists believe that > 65% of the bacterial population in water distribution systems are in the VBNC state. The traditional plate counts used to test water quality often represent < 1% of the total bacteria present in environmental samples.

VBNC bacteria can be resuscitated (brought back to their normal state during which they can be cultured under laboratory conditions) by co-culture with FLA.

Slide 16: Biofilms

Microorganisms form biofilms on surfaces which provide another source of protection against water treatment regimes. Free living amoebae use the bacteria in these biofilms as a source of nutrition. Bacteria and other organisms within the biofilm are protected by a slime layer which prevents biocides from reaching the bottom layers of the biofilm, thereby reducing the effects of water treatment programmes.

Slide 17: Background to research program

Infectious diseases are a global hazard that puts every nation and every person at risk. The recent cholera and swine flu outbreaks are examples of how fast and wide the effects of infectious diseases can be. Most countries now realise that the spread of infectious disease is a global problem with huge economic and humanitarian consequences.

It is currently believed that about 1000 people die (worldwide) every HOUR from waterborne infections. This situation may worsen in many parts of the world because pathogens can remain in reservoir hosts (eg. amoebae) or the environment for long periods of time. This poses a constant threat to human health.

In addition, a breakdown of preventative measures (for example reduced chlorine levels and poor water filtration) can increase this threat considerably.

The current limitations in surveillance and the non-availability of diagnostic tests increase the threat of waterborne infectious diseases even more, especially in South Africa.

Slide 18: Reason for study

With this in mind the NIOH has recently embarked on a new research focus area, namely the presence of amoeba resistant bacteria in water distribution systems. The next slide explains the program in more detail.

Slide 19: Illustration of ARB program

The ARB program will be conducted in 5 distinct phases.

Phase I is currently underway. We are in the process of setting up and validating the methods for isolation of amoeba resistant bacteria. The NIOH is collaborating with the Tshwane University of Technology on this part of the project, during which one student will obtain a M. Tech degree. So far we have not been able to obtain funding but this should soon be changed. We are using the international standard method for validating microbiological methods and are currently about half way through the study. We have tested the methods on water samples from a water treatment plant and the results are promising.

During Phase II water and biofilm samples will be tested for the presence of ARB, again in collaboration with academic institutions and industry. Once the prevalence of ARB in these systems have been established, worker health in the institutions where ARB are most likely to occur will be tested by way of clinical samples. Phase IV (biocide resistance and bio-aerosol formation) and Phase V (setting up a reference facility and training laboratories to conduct these experiments) will be developed once we know whether these organisms actually do pose a health threat to workers and ultimately the general public.

Slide 20: Explanation

Self-explanatory

Slide 21: Statistics SA data

I compared causes of death from 1997 – 2003 as described in the Statistics SA report on Mortality and Causes of Death in South Africa (1997 – 2003). I looked at the figures for TB, "influenza and pneumonia", chronic lower respiratory diseases" and HIV/AIDS and saw the following.

The red line represents TB deaths and the blue line, deaths from IP (please note that these figures do not include deaths from the current avian flue pandemic). The yellow and green lines represent deaths from CLRD and HIV/AIDS respectively.

All I want to emphasize is that the death rates for TB and IP are steadily rising. TB deaths increased from 6.8% in 1997 to a current figure of just over 12% of the total death rate. IP increased from 3.6% in 1997 to 6.4% in 2003 (in other words, it doubled)

Slide 22: Environmental Protection Agency report

According to the Environmental Protection Agency (EPA) of the USA's latest report, the assessment and evaluation of detection methods should be seen as an integral and essential component in the assessment of public and occupational health significance, occurrence/ecology and treatment of emerging pathogens. The areas of specific concern mentioned in the report (get reference) that need to be studied also include:

- Sample collection and analytical methods;
- The occurrence and distribution of emerging pathogens in water and biofilm;
- Routes of exposure;
- Susceptibility of the human populations and the microbial dose necessary to cause disease and resistance to treatment;
- Assessment of public- and occupational health significance of various emerging pathogens;
- Assessment of the occurrence and ecology of pathogens in potable and non-potable aquatic environments;
- Assessment of the efficacy of conventional water treatment practices in removal or inactivation of pathogens.
- Following up on a high-profile 1992 report from the Institute of Medicine, Microbial Threats to Health examines the current state of knowledge and policy pertaining to emerging and re-emerging infectious diseases from around the globe. It examines the spectrum of microbial threats, factors in disease emergence, and the ultimate capacity of the United States to meet the

challenges posed by microbial threats to human health. From the impact of war or technology on disease emergence to the development of enhanced disease surveillance and vaccine strategies, Microbial Threats to Health contains valuable information for researchers, students, health care providers, policymakers, public health officials, and the interested public.

Slide 23: South Africa – Water Research Commission

Previous reports have shown several gaps in previous SA water research. The following were emphasised:

- Detection methods need improvement
- Impact studies must be done (especially concerning vulnerable groups including HIV/AIDS, TB, immuno-compromised, children, old people, where living conditions not good)
- There is a desperate need for a national surveillance system for water-associated disease
- And burden of disease investigations at provincial level
- There is also a lack of technical guidelines in the water industry
- It was also found that knowledge and technology transfer need special attention

Slide 24: South Africa - industry

Similarly, discussions with role players in industry emphasized the following questions:

- Are ARB present in water distribution systems and if they are present, are they in the planktonic (free flowing) or sessile (attached to surfaces) phase?
- Which ARB are present?
- Can they be transported through aerosolisation?
- If this is the case, are the particles small enough to be inhaled?
- How does this relate to South African legislation?

Slide 25: Previous South African studies

Results from previous South African studies indicated the presence of Legionella in 82% of industrial waters tested – in 54% of these samples the level of Legionella bacteria present was very high. In addition, pneumonia cases occur often, especially under mine workers, but in most cases the etiologic agent cannot be identified using conventional laboratory methods. It was found that co-culture of these samples with ARB improve culturability of the organisms.

Slide 26: Impact and conclusion

It is hoped that this study will answer at least some of the questions regarding the prevalence of amoeba resistant bacteria in waters from various sources and their risk to the health of workers and the general public.

The study will have an impact in the water treatment industry (studies of biocide resistance), the medical and pharmaceutical industries (resistance to antibiotics may be addressed if the etiologic agent is known), and in the design and maintenance of ventilation and water distribution systems.