CONTROL OF PSEUDOMONAS AND OTHER PATHOGENS IN SATURATION CHAMBERS
**PSEUDOMONAS AERUGINUSA (“PYO”)**

Metabolic products
- Urine
- Faeces
- Sweat
- Dust (dead skin)

“Introduced” products
- Foodstuff
- Books, paper etc
- Fresh water (drinking & sanitary)
- Seawater
- Mud etc

Chamber environment
- High PPO₂
- High temperature
- High humidity
- Recycled gas
- Close personal contact
PSEUDOMONAS AERUGINOSA (“PYO”)

- PSEUDOMONAS SPECIES > 190
- AEROBIC
- VERY MOBILE
- UBIQUITOUS
- OPPORTUNISTIC
- THRIVES WHERE CARBON SOURCE IS AVAILABLE
- GRAM NEGATIVE
➢ TWO MAIN CATEGORIES OF BACTERIA
➢ DEPENDS ON REACTION TO CRYSTAL VIOLET STAIN
➢ STAINING INVOLVES APPLICATION OF CRYSTAL VIOLET AND THEN DECOLOURING WITH A COUNTER-STAIN E.G SAFRANIN
➢ GRAM-POSITIVE BACTERIA WILL RETAIN THE CRYSTAL VIOLET
➢ WHILE
➢ GRAM-NEGATIVE BACTERIA WILL LOSE VIOLET DYE COLOUR AND TAKE UP THE COUNTER STAIN COLOUR (RED/PINK)
STRUCTURE OF BACTERIAL CELL WALL Dictates REACTION TO STAINING

GRAM-NEGATIVE HAVE AN ADDITIONAL LAYER OF LIPOPOLYSACCHARIDE WHICH PREVENTS STAIN TAKE-UP

THIS LAYER CAN INCREASE RESISTANCE TO ANTIBIOTICS
GRAM-POSITIVE BACTERIA

Gram positive

Cocci

Staphylococcus
Catalase +

- Coagulase -
  - S. epidermidis
    - Novobiocin sensitive
  - S. saprophyticus
    - Novobiocin resistant

S. aureus
Coagulase +

Streptococcus
Catalase -

- β-hemolytic (clear)
- γ-hemolytic
- α-hemolytic (green)

bacilli
Carynebacterium
Clostridium
Listeria
Bacillus
Additional layer in cell wall protects against certain antibiotics (e.g. Penicillin) and detergents/disinfectants

However, antibiotics have been developed which will attack gram-negatives (e.g. Ampicillin, Chloramphenicol, Streptomycin)
“INTRODUCED” PRODUCTS
- FOODSTUFF
- BOOKS, PAPER ETC
- FRESH WATER
- SEAWATER
- MUD ETC

DIVERS!

FRESH (SANITARY) WATER

BIOFILMS IN WATER SUPPLY SYSTEMS.......

Legionella pneumophila
in Norwegian naval vessels

BACKGROUND: The emergence of Legionella pneumophila in water supply systems is a known issue. One way to identify occurrences of L. pneumophila in the water supply system is found Norwegian naval vessels as the basis for framing preventive measures against legionella infection.

MATERIAL AND METHODS: Water samples were collected from identical installations and from the water distribution network on board the vessels and from two water intake "hot spot" stations. The sampling taking place was monitored and preserved to an area size of 1 cm² for a period of 4 weeks. The samples were subjected to analysis, including culturing and identifying, with a new to identify the presence of L. pneumophila and to determine the source.

RESULTS: Legionella pneumophila was detected in 4 of the 5 vessels in the first round of sampling. In 3 vessels, pneumophila was not detected in the second round. The same samples were analyzed in all vessels, and the presence of L. pneumophila was identified in all vessels. The same samples were analyzed in all vessels, and the presence of L. pneumophila was identified in all vessels.

INTERPRETATION: Legionella pneumophila was not present in active biofilms, but it was present in the biofilms. The same samples were analyzed in all vessels, and the presence of L. pneumophila was identified in all vessels.

The Legionella pneumophila was found in both small and large communities. The same samples were analyzed in all vessels, and the presence of L. pneumophila was identified in all vessels.

The Legionella pneumophila was found in the water supply system of approximately 50% of Norwegian naval vessels. The same samples were analyzed in all vessels, and the presence of L. pneumophila was identified in all vessels.

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BACTERIAL FORMS IN WATER SUPPLIES

- FREELIVING (PLANKTONIC)

- INTRACELLULAR

- BIOFILM
THE TERM “BIOFILM” DESCRIBES ANY ACCUMULATION OF MICROORGANISMS ADHERING TO INTERFACES (FREQUENTLY: SOLID/LIQUID). THE ORGANISMS IN A BIOFILM ARE EMBEDDED IN A MATRIX OF EXTRACELLULAR POLYMERIC SUBSTANCES (EPS).
Bacteria colonise inner surfaces of the drinking water installation and build up biofilms.

Produce “glue”, Extracellular Polymeric Substance - EPS

Quorum sensing. Higher levels of homoserine lactones – HSL Communication.....

Genetic changes and increased resilience

Biofilm particles can shear off and contaminate water for drinking & washing
TOP LAYER MAY GET KILLED OR SLOUGHED OFF DEPENDING ON RESILIENCE

LOWER LEVELS CONTINUE TO THRIVE OR PROTECT THEMSELVES BY GOING INTO A DORMANT STATE OR FORM HARD COATING (CYST) OR VIABLE BUT NOT CULTURABLE PHASE (VBNC)

“REACTIVATE” WHEN CONDITIONS RETURN TO NORMAL
TRIALS

- DECIDED TO PERFORM SOME TRIALS ON 2 SHIPS
Legionella spp. Samples Before and After Filter

Before Filter

Mean 2769 cfu/litre
**Legionella spp. Samples Before and After Filter**

Before Filter:
- Mean: 2769 cfu/litre

After Filter:
- Mean: 613 cfu/litre
Before Filter

After Filter

Pseudomonas aer. Samples Before and After Filter

Mean 85 cfu/100ml
**TRIALS VESSEL 1**

**Pseudomonas aer. Samples Before and After Filter**

- **Before Filter**
  - Mean: 85 cfu/100ml

- **After Filter**
  - Mean: 60 cfu/100ml
SAMPLING TECHNIQUES

➢ SAMPLING TECHNIQUES EXTREMELY IMPORTANT, E.G. ENTRAINMENT, CONTACT WITH SAMPLE BOTTLE ETC

- FALSE POSITIVES
Legionella spp. Samples Before and After Filter
Legionella spp. Samples Before and After Filter

Before Filter

After Filter

Negative

Positive
**Pseudomonas aer.** Samples Before and After Filter

**Mean**

39 cfu/100ml

- **Before Filter**
- **After Filter**
Pseudomonas aer. Samples Before and After Filter

Mean 39 cfu/100ml
Total Viable Count Samples Before and After Filter

Mean 922 cfu/100ml
TRIALS VESSEL 2 - IMPROVED SAMPLING

Total viable Count Samples Before and After Filter

Mean 922 cfu/100ml

Before Filter

After Filter

Negative

Positive
POINT OF USE FILTERS
CHALLENGES FOR FILTERS
ANALYSIS OF FILTER CONDITION

Control

After 3 weeks
In DDC

#2635  2014/01/15  15:50 HL  D6.7  x500  200 um

#2642  2014/01/15  16:13 HL  D7.8  x500  200 um
**ANALYSIS OF FILTER CONDITION**

**Spectrum: Point**

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ROUTINE PREVENTATIVE MEASURES

STRICT PERSONAL HYGIENE- “WASH TOP – DOWN”!

GOOD CHAMBER HOUSE-KEEPING

FREQUENT BEDDING CHANGES

NO SHARED HEADPHONES

NO COTTON BUDS!

OWN HAT LINER

PROPHYLACTIC (PREVENTATIVE) EAR DROPS. ONE BOTTLES FOR EACH EAR PER DIVER (LABELLED). ALUMINIUM ACETATE IN ACETIC ACID

SHOWER AND TAP FILTERS
SWABBING

➢ SWAB AND YOU WILL FIND!

➢ ROUTINE AND WIDESPREAD SWABBING IS OF LITTLE USE

➢ SWABBING OF TOILETS, SINK DRAINS, BILGES ETC. WILL GIVE POSITIVE RESULTS (SAME AS IT WOULD AT HOME!)

➢ “JUDICIAL” SWABBING CAN BE VERY USEFUL

➢ SHOWER HEADS
➢ TAPS
➢ SUSPECTED INFECTION (BEFORE TREATMENT DROPS)
➢ ALL SURFACES IN A POTABLE WATER SYSTEM, IN CONTACT WITH WATER WILL BE COLONISED BY BIOFILM.

➢ PLUMBING MATERIALS CAN BE NUTRIENTS FOR THE BACTERIA IN THE BIOFILM.

➢ CULTURE OF SAMPLES MAY NOT REVEAL THE EXTENT OF CONTAMINATION

➢ RESULTS OF SAMPLES FROM SAME SOURCE CAN DIFFER WIDELY E.G. SAMPLES FROM SAME TAP ONE HOUR APART
  <1 cfu/100ML
  > 100 cfu/100ML

➢ AMOUNT OF BACTERIA GROWN IN CULTURE MAY BEAR VERY LITTLE RELATIONSHIP TO ACTUAL LEVELS AS MANY BACTERIA ARE “VIABLE BUT NOT CULTURABLE” – VBNC.

➢ HEAT TREATMENT AND DISINFECTION HAVE TEMPORARY EFFECTS, STRESSED CELLS MAY GO IN TO VBNC STATE AND THEN “REACTIVATE” WHEN STRESS CONDITIONS HAVE GONE, ANY DEAD CELLS PROVIDE NUTRIENT FOR THE SURVIVORS AND NEWCOMERS
SUMMARY

➢ BIOFILM POPULATIONS ADAPT AND CAN INCREASE THEIR RESISTANCE TO DISINFECTANTS AND ANTIBIOTICS

➢ IT IS VIRTUALLY INEVITABLE THAT THE CHAMBER WATER SUPPLY SYSTEMS WILL BE POPULATED BY PSEUDOMONAS AND OTHER BACTERIA

➢ POINT OF USE FILTRATION (POU) IS RECOGNISED BY REGULATORS AS THE MOST EFFECTIVE DEFENCE IN WATER SUPPLY SYSTEM

➢ HENCE WIDELY USED IN HOSPITAL ICU/HDU, PAEDIATRIC UNITS, CANCER TREATMENT CENTRES AS WELL AS WASHING FACILITIES IN HEALTH CLUBS, GYMS ETC.

➢ LIFESPAN OF FILTERS REDUCED DUE TO POOR WATER QUALITY ON INLET SIDE

➢ CAN BE AN IMPORTANT TOOL IN REDUCING INFECTION RISK IN DIVERS.