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EMERGING PATHOGENS OF CONCERN IN HEALTHCARE SETTINGS

Those in charge of managing healthcare environments have had guidance on the importance of controlling the presence of *Legionella*, via the Health Technical Memorandum (HTM) 04-01 ⁽¹⁾ which provided specific guidance for the control of *Legionella* in hot and cold water systems in healthcare settings. The addendum to HTM 04-01 ⁽²⁾ was published in March 2013 in response to the threat posed to immunocompromised patients in augmented care settings by *Pseudomonas aeruginosa*. However, there are other emerging pathogens of concern that should be considered by Healthcare and Water Treatment companies; these are known as the ESKAPE pathogens. The ESKAPE pathogens were first identified by the Infectious Diseases Society of America (IDSA) in 2004 ^(3, 20). Recently *S. maltophilia* has become more prevalent, leading to ALS Environmental producing a specific suite of analysis for the IDSA pathogens plus *S. maltophilia* ^(4, 5), referred to as ESKAPES pathogens:

<u>ESKAPES Pathogen</u>	<u>General Description</u>
<i>Enterococcus faecium</i>	Belonging to the <i>Enterococci</i> genus, <i>E. faecium</i> are Gram-positive cocci that can grow in both aerobic and anaerobic conditions ⁽⁶⁾ , in the presence of bile salts or sodium azide; these are conditions that are inhibitory to the vast majority of Gram-negative bacteria. <i>E. faecium</i> can survive in temperatures up to 44°C.
<i>Staphylococcus aureus</i>	Gram-positive non-motile cocci occurring found as single cells or clumps of cells. <i>Staphylococcus spp.</i> are anaerobic bacteria. The <i>Staphylococci</i> bacteria are spread through the air on dust particles and are resistant to drying. <i>Staphylococci</i> grow between 30 and 37°C and can also be found in water and human faeces ⁽⁶⁾ . Causing fibrin to coagulate, <i>Staphylococcus aureus</i> are oxidase negative, catalase positive which produce coagulase. Three species of <i>Staphylococci</i> form part of the normal flora of the skin and mucous membranes of humans: <i>S. epidermis</i> and <i>S. haemolyticus</i> and <i>S. aureus</i> have all been linked with human infections.
<i>Klebsiella pneumoniae</i>	A family member of the Enterobacteriaceae, <i>Klebsiella spp.</i> are non-motile Gram-negative bacteria and are a part of the coliform group of organisms. Capable of growth at 37°C <i>Klebsiella</i> has a range of species including <i>K. pneumoniae</i> , <i>K. oxytoca</i> , <i>K. planticola</i> and <i>K. terrigena</i> . A large polysaccharide capsule distinguishes <i>Klebsiella</i> from other coliform and <i>Enterobacteriaceae</i> family members.
<i>Acinetobacter baumannii</i>	<i>Acinetobacter</i> is a group of bacteria commonly found in soil, water and sewage environments. <i>Acinetobacter spp.</i> are part of the natural microbial flora of the skin and occasionally the respiratory tract of healthy individuals



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	<p>^(6,9). A Gram-negative coccobacillus and rapidly emerging pathogen in health care settings. <i>A. baumannii</i> is usually introduced into a hospital by a colonized patient, due to its ability to survive on artificial surfaces and resist desiccation it can survive and potentially infect new patients.</p> <p>There are many species of <i>Acinetobacter</i> that can cause human disease ⁽⁶⁾, <i>A. baumannii</i> accounts for about 80% of reported infections. It is suspected that <i>A. baumannii</i> growth favors nosocomial settings due to the constant use of antibiotics by patients in the hospital ⁽¹⁰⁾.</p>
<i>Pseudomonas aeruginosa</i>	<p>An aerobic Gram-negative bacteria that is a member of the family <i>Pseudomonadaceae</i>. The bacteria frequently colonise man made environments, such as water systems, where they contribute to the production of Biofilms, which some commentators suggest provide the breeding ground for Legionella bacterial growth.</p> <p>Presumptive <i>Pseudomonas aeruginosa</i> produce pigmented (green brown or reddish brown) and/or fluorescent colonies on <i>Pseudomonas</i> C-N agar after incubation at 37°C for 40 - 48 hours ⁽²⁾.</p>
<i>Enterobacter</i> Species	<p>A common Gram-negative anaerobic non-spore-forming bacteria, <i>Enterobacter</i> is a member of the <i>Enterobacteriaceae</i> family, with several pathogenic strains ⁽⁶⁾. Three clinically important species from this genus are <i>E. aerogenes</i>, <i>E. cloacae</i> and <i>E. sakazaki</i>., which has been found as a contaminant in infant formulas. <i>E. sakazaki</i> has been found to be more resistant to osmotic and dry stress than other members of the <i>Enterobacteriaceae</i> family.</p> <p><i>Enterobacter</i> species are biochemically similar to <i>Klebsiella</i>; unlike <i>Klebsiella</i>, however, <i>Enterobacter</i> is ornithine positive.</p>
<i>Stenotrophomonas maltophilia</i>	<p>Difficult to treat once in a human host, due to its antibiotic resistant nature, <i>S. maltophilia</i> is a Gram-negative bacteria and an opportunistic human pathogen ^(4,5, 7, 8, 10). In nosocomial settings <i>S. maltophilia</i> bacteria can result in infections due to its high tolerance to antibiotics. Although infection is rare, it is difficult to treat and the organism has shown the capacity to quickly develop into strains with increased drug resistance.</p>

The IDSA report cited the antibiotic resistant nature of the ESKAPE bacteria as the main consideration for concern, which is of concern as drug companies are reducing funding into the development of new antibiotics ⁽³⁾. The ESKAPES pathogens are of more prominent concern in healthcare settings due to the high susceptibility of patients (old, young and immunocompromised) to infection.

ESKAPES: Stability Times

All of the ESKAPES pathogens have a sample stability time of 24 hours from the point of sampling for accredited analysis. This means that the sample needs to be sampled, transported to the laboratory, registered and processed within that time period. The guidance for stability times is provided by United Kingdom Accreditation Service (UKAS) ⁽¹¹⁾ with laboratories able to extend stability times with sufficient validated data.



ESKAPES: MALDI-ToF Confirmations

The use of Matrix Assisted Laser Desorption and Ionisation by Time of Flight (MALDI-ToF) has been well documented for the rapid identification of bacteriological isolates⁽²¹⁾. The innovative approach developed by ALS Environmental allows our microbiologists to provide an instant confirmation of any isolates from culture media, allowing us to provide results back to our customers along with Colony Forming Unit (CFU) by volume filtered. The technique generates a mass spectra creating a protein fingerprint unique to each genus and species of bacteria. ALS Environmental then compare the protein finger print generated by the MALDI-ToF against an ever expanding bespoke internal library of over 15,000 known species and genus of over 1,000 bacteria.

The HTM 04-01 and the addendum both state that the confirmation of *Legionella* and *Pseudomonas aeruginosa* should be performed at a UKAS ISO 17025:2005 accredited laboratory using referenced methods. The use of MALDI-ToF to confirm the pathogens protein finger print is in line with all UKAS, HTM and Drinking Water Testing Specification (DWTS) guidance and enables ALS Environmental to significantly reduce the confirmation time for all ESKAPES and *Legionella* positive samples:

ESKAPES Pathogen	Incubation Time	Traditional Confirmation:	MALDI-ToF Confirmation:	MALDI-ToF Saving
<i>Enterococcus faecium</i>	2 days	1 day	Minutes	1 day
<i>Staphylococcus aureus</i>	2 days	1 day	Minutes	1 day
<i>Klebsiella pneumoniae</i>	1 day	1 day	Minutes	1 day
<i>Acinetobacter baumannii</i>	1 day	1 day	Minutes	1 day
<i>Pseudomonas aeruginosa</i>	2 days	1 day	Minutes	1 day
<i>Enterobacter spp.</i>	1 day	1 day	Minutes	1 day
<i>Stenotrophomonas maltophilia</i>	1 day	1 day	Minutes	1 day

The MALDI-ToF also allows ALS Environmental to identify the other strains of the ESKAPES pathogens that are at a lower risk to the immunocompromised, allowing infection control to take the appropriate action in an efficient and effective manner.



Enterococcus faecium ^(6, 12,15)

Source

The faeces of warm blooded animals are a common source of Enterococci bacteria and are an indicator of contamination, with *Coliforms* and *E. coli* being the primary indicators. *Enterococci* are more resilient than the primary bacteria to environmental stress and chlorination, they tend to outlive their counterparts outside of laboratory conditions.

By using the MALDI-ToF confirmation technique ALS Environmental are able to identify 34 different species of *Enterococcus*, with 10 different strains of *E. faecium*. This allows our customer to distinguish between the contamination type and therefore, potentially the source of contamination. *E. faecalis*, *E. faecium* and *E. durans* are normally present in the faeces of humans and various animals. *Streptococcus bovis*, *Streptococcus equinus* and *E. avium* have been found to be associated with cattle, horse and bird faecal contamination respectively. These organisms are not normally found in human faeces.

Pathogenesis and Routes of exposure

The *E. faecium* pathogen enters the human body through contact (ingestion, open wounds etc.) with contaminated sources. Once infected, the patient suffers from septicaemia and the mortality rate can be as high as 50%.

Significance in water

The presence of intestinal *Enterococci* provides evidence of recent faecal contamination and detection should lead to consideration of further action, which could include further sampling and investigation of potential sources, such as inadequate treatment or breaches in distribution system integrity.

Staphylococcus aureus ^(6, 13)

Source

Staphylococci bacteria can colonise in water and soil; moreover the bacteria are part of human flora and commonly colonise the skin and mucous membranes e.g. the naso pharyngeal system.

Pathogenesis

S. aureus bacteria can multiply and colonise a patient in a short time frame, especially so in the immunocompromised where a weakened immune system and normal body temperature provides the ideal conditions for growth. This can result in a range of ailments including boils, skin sepsis, post-operative wound infections, enteric infections, septicaemia, endocarditis, osteomyelitis, pneumonia, impetigo, meningitis and arthritis.

Routes of exposure

The most common pathway for *Staphylococcus* infection is via skin to skin contact, with inadequate basic hand hygiene, from staff, patients and visitors being a frequent root cause in nosocomial settings. Food contaminated with *S. aureus* can provide an ideal breeding ground for



the bacteria, especially when left at room temperature. The consumption of foods containing *S. aureus* toxins can lead to enterotoxin food poisoning within a few hours.

Significance in drinking water

Although *S. aureus* can occur in drinking-water supplies, there is no evidence of transmission through the consumption of such water. Although staphylococci are slightly more resistant to chlorine residuals than *E. coli*, their presence in water is readily controlled by conventional treatment and disinfection processes. ALS Environmental can identify over 40 different species of *Staphylococcus*. 14 of these species are strains of *S. aureus*; 10 of which are variants on the sub species *S. aureus* sub species *anaerobius* and *S. aureus* sub species *aureus*.

Klebsiella pneumoniae ^(6, 14, 15)

Source

Klebsiella colonisation can occur in soil, water or animal faeces. The bacteria are more prominent in waters that are rich in nutrients. In nosocomial scenarios the bacteria have been known to colonise taps and water distribution systems.

Pathogenesis

Poor hand hygiene of staff, patients and visitors has been identified as one of the main sources of colonisation of *Klebsiella spp.* in nosocomial settings. The weakened immune systems of the immunocompromised, both young and old, or those with open wounds are highly susceptible to infection from *Klebsiella* and colonisation may lead to infection. On rare occasions, *Klebsiella spp.*, notably *K. pneumoniae* and *K. oxytoca*, may cause serious infections, such as destructive pneumonia.

Significance in water

The contamination of drinking water with *Klebsiella* is not considered as a potential source of gastrointestinal illness. The bacteria are generally present in biofilms and are unlikely to represent a human health risk. The pathogen is usually well controlled with chemical disinfection and entry into

distribution systems can be prevented by adequate treatment. ALS Environmental hold 3 different species of *Klebsiella* and 3 *Raoultella species*, *Raoultella* and *Klebsiella* are very closely related, with some *Raoultella* being reclassified as *Klebsiella* in some circumstances by older traditional microbiological confirmation techniques. The MALDI-ToF database used and developed by ALS Environmental holds 13 strains of *K. pneumoniae* which we are able to uniquely distinguish; offering a distinct advantage over the traditional confirmation approach.

Acinetobacter baumannii ^(6, 10, 16, 17)

Source

Acinetobacter spp. can survive for an extended period on most surfaces. The bacteria particularly favours nosocomial settings due to the high use of antibiotics, as outlined in the IDSA 2004 and



WHO 2011 paper and guidance respectively. The MALDI-ToF confirmation technique used by ALS Environmental holds 19 different species of the genus *Acinetobacter*, 15 of which are *A. baumannii*. The pathogen can colonise soil, water, sewage and the skin of healthy people. This allows the bacteria to be spread by skin contact from person-to-person or from a colonised surface to another person.

Routes of exposure

Intravenous catheters have been identified as a source of infection in patients with *Acinetobacter*. The WHO 2011 guidance on Drinking Water lists *Acinetobacter* as a pathogen which transmission through Drinking Water has been suggested but not yet been conclusively evidenced.

Pathogenesis

Acinetobacter infections in immunocompromised individuals can include urinary tract infection, pneumonia, meningitis and wound infection. There are various reports of veterans from the US and UK, who were injured while on tours of Iraq or Afghanistan being colonised by *A. baumannii*; the evidence suggest that the increase in infection, leading to the pathogen being referred to in many quarters as “Iraqibacter”.

Significance in water

Acinetobacter spp. are frequently detected in treated drinking-water supplies however an association between the presence of *Acinetobacter spp.* in drinking-water and clinical disease has not been confirmed. There is no evidence of gastrointestinal infection through ingestion of *Acinetobacter spp.* in drinking-water among the general population. However, transmission of non-gastrointestinal infections by drinking-water may be possible in susceptible individuals, particularly in settings such as health-care facilities and hospitals.

Pseudomonas aeruginosa ^(2, 6, 15, 18)

Source

P. aeruginosa is a common environmental organism and can be found in faeces, soil, water and sewage. It can multiply in water environments and also on the surface of suitable materials in contact with water. It has been isolated from a range of moist environments such as sinks, water baths, hot water systems, showers and spa pools. The unique MALDI-ToF database utilised by ALS Environmental has over 70 different *Pseudomonas spp.*; 9 of which are *P. aeruginosa*.

Pathogenesis

Principal infections include septicaemia, skin, respiratory, urinary tract and ear and eye . These infections may occur due to burns, surgery and open wounds. Cystic fibrosis and immunocompromised patients are prone to colonisation with *P. aeruginosa*, which may lead to serious progressive pulmonary infections.

Routes of exposure

The highly susceptible nosocomial patients include those on breathing machines, premature babies and patients with wounds from surgery or from burns. Additionally, healthy people can



also develop mild illnesses with *P. aeruginosa*, especially after exposure to water. Ear infections, especially in children, and more generalised skin rashes may occur after exposure to inadequately chlorinated hot tubs or swimming pools.

Significance in water

P. aeruginosa can be found in drinking water and can lead to potential colonisation in production and healthcare facilities. Bottled waters should also be examined for this organism. *P. aeruginosa* also occurs in swimming pools and bathing facilities which may be a particular concern for augmented care units due to the organisms antibiotic resistant nature. Large numbers of this bacterium growing in polluted waters, swimming pool waters or spa pool waters may, following immersion, produce ear infections or a follicular dermatitis.

Enterobacter species ^(6,10, 14, 15)

Source

Enterobacter spp. are present in the environment (such as soil and water), sewage. Additionally, *Enterobacter spp.* are present in animals and humans, where it can cause opportunistic infections within the gastrointestinal system. The MALDI-ToF is able to identify 12 different species of *Enterobacter* and has 41 different strains.

Pathogenesis

E. sakazakii has been associated with sporadic cases or small outbreaks of sepsis, meningitis, cerebritis and necrotizing enterocolitis. Most of the infections are seen in low-birth-weight infants (i.e. less than 2 kg) or infants born prematurely (i.e. less than 37 weeks of gestation). Mortality has been reported to be as high as 50% but has decreased to less than 20% in recent years.

Routes of exposure

Risk factors for nosocomial *Enterobacter* infections include hospitalization of greater than 2 weeks, invasive procedures in the past 72 hours, treatment with antibiotics in the past 30 days, and the presence of a central venous catheter. Specific risk factors for infection with nosocomial multidrug-resistant strains of *Enterobacter spp.* include the recent use of broad-spectrum cephalosporins or aminoglycosides.

Significance in water

Enterobacter is present in water, although its presence can be prevented with a successful water treatment programme. In particular, the *E. sakazakii* strain is sensitive to disinfectants, and its presence can be prevented by adequate treatment.

Stenotrophomonas maltophilia ^(4, 5, 6, 7, 8)

Source

S. maltophilia is an organism of low virulence and frequently colonises fluids used in the hospital setting (eg, irrigation solutions, intravenous fluids) and patient secretions (eg, respiratory



secretions, urine, wound exudates). *Stenotrophomonas* can be found in soils, waters and has been known to colonise drinking water systems. Although it has no natural infection route to humans *S. maltophilia* infection can be transmitted by contaminated prosthetics such as catheters and intravenous lines. ALS Environmental are able to identify 4 of the 12 species of *Stenotrophomonas*, including *S. maltophilia*, for which we hold 9 different strains of in our MALDI-ToF database.

Pathogenesis

In severely ill patients, *S. maltophilia* causes a wide range of infections such as bacteremia, pulmonary infections, urinary tract infections, wound infections, meningitis and endocarditis. Infection risk of opportunistic pathogens such as the *maltophilia* species of *Stenotrophomonas* can be elevated in healthcare settings and other environments where immunocompromised patients, such as in care homes or Intensive Care Units (ICU) may be exposed.

Significance in water

The presence of *S. maltophilia* in the water of nosocomial settings is a concern to infection control due to its opportunistic and antibiotic resistant nature. The risk of *S. maltophilia* infection comes from the bacteria's ability to survive on artificial surfaces and be transferred, via medical equipment, to immunocompromised patients. Once an immunocompromised patient is infected with *S. maltophilia* the bacterial infection can be difficult to treat and can result in severe infection.

Summary

The ESKAPE pathogens as identified by the IDSA ⁽³⁾ in 2004 still pose a threat to the immunocompromised and healthcare institutions; with *S. maltophilia* being considered a further potential risk factor due to the pathogens antibiotic resistance. When analysing water samples for any pathogenic or bacteriological contamination time is aessence important factor. Infection Control teams need as much information on any potential risk factors, such as water borne pathogens, as soon as possible. MALDI-ToF allows for rapid confirmed and identified analytical data down to both the genus and the species of bacteria (and or fungi).

The risks associated with the ESKAPES pathogens are not going away. As our use of antibiotics increases the health risks posed to the patients and the general population of these pathogens is also likely to increase. With a stringent Water Safety Group, well maintained maintenance plans and routine analytical testing and monitoring we can help keep patients, staff and the general public safe from Legionella and ESKAPES pathogens.



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