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# Development and evaluation of a new PCR assay for detection of *Pseudomonas aeruginosa* D genotype

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## ABSTRACT

This report describes a new PCR-based assay for the detection of *Pseudomonas aeruginosa* genotype D in occupational saturation diving systems in the North Sea. This genotype has persisted in these systems for 11 years (1993–2003) and represents 18% of isolates from infections analysed during this period. The new PCR assay was based on sequences obtained after randomly amplified polymorphic DNA (RAPD)-PCR analysis of a group of isolates related to diving that had been identified previously by pulsed-field gel electrophoresis (PFGE). The primer set for the D genotype targets a gene that codes for a hypothetical class 4 protein in the *P. aeruginosa* PAO1 genome. A primer set able to detect *P. aeruginosa* at the species level was also designed, based on the 23S-5S rDNA spacer region. The two assays produced 382-bp and 192-bp amplicons, respectively. The PCR assay was evaluated by analysing 100 *P. aeruginosa* isolates related to diving, representing 28 PFGE genotypes, and 38 clinical and community *P. aeruginosa* isolates and strains from other species. The assay identified all of the genotype D isolates tested. Two additional diving-relevant genotypes (TP2 and TP27) were also identified, as well as three isolates of non-diving origin. It was concluded that the new PCR assay is a useful tool for early detection and prevention of infections with the D genotype.

**Keywords** Genotypes, identification, infections, PCR assay, *Pseudomonas aeruginosa*, saturation diving

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## INTRODUCTION

Saturation diving is used regularly during the maintenance and inspection of offshore subsea petroleum production systems in the North Sea. The working and living environment of the divers is unique, in that they are chronically exposed to hyperbaric, warm and humid surroundings that are capable of maintaining a rich microbial flora [1–5]. Exposure to such hyperbaric environments has been associated with a risk of acquiring various conditions, including decompression sickness, arterial gas embolism, neurological symptoms and pulmonary dysfunctions [6]. However, the medical problems encountered most frequently by divers are ear, nose and throat complications [7]. Of these, acute infection of the outer ear has been a well-known and frequent problem associated with saturation diving since the introduction of the technique in the late 1960s. Outbreaks of infection involving several divers have been a frequent cause of costly interruptions in operations in both the USA and the UK [8,9].

A specific cause of these outbreaks is *Pseudomonas aeruginosa*, which has also long been known as the predominant bacterial pathogen in 'swimmer's ear' [10]. *P. aeruginosa* is a common bacterium that occurs worldwide in both fresh and seawater, in soil and on plants. The organism is characterised by its metabolic versatility and its exceptional ability to adapt to and colonise various ecological niches [11]. *P. aeruginosa* is a well-known opportunistic pathogen and is of growing importance in both hospital and community-acquired infections [12].

Field research on the presence of *P. aeruginosa* in infections and the environment has been continuous since the 1980s during the

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exploration of the Norwegian petroleum sector. Systematic analysis of isolates by biotyping and pulsed-field gel electrophoresis (PFGE) during an 18-year survey has produced evidence of the involvement of specific *P. aeruginosa* genotypes in skin infections among occupational saturation divers in the Norwegian sector [3–5]. Some *P. aeruginosa* genotypes have been detected more frequently than others in single infections and recorded outbreaks [3,4]. These genotypes have been shown to persist in saturation diving systems for years, despite the fact that new genotypes are being introduced continuously [4,5]. Genotype D, which is the most prominent of these genotypes, has been isolated from single, recurrent and clusters of infections involving several different genotypes and divers [4].

For efficient control and prevention of *P. aeruginosa* infections and a better understanding of their route(s) of transmission in operational saturation diving systems, rapid and sensitive tools for detection of the genotypes involved in infections are of crucial importance. This study describes the development of a PCR-based assay for detection of *P. aeruginosa* genotype D in occupational diving environments, as well as for the identification of *P. aeruginosa* in general. Randomly amplified polymorphic DNA (RAPD)-PCR analysis was used to verify the PFGE genotypes identified previously and formed the basis for the design of the new PCR assay.

## MATERIALS AND METHODS

### Bacteria and samples

A group of *P. aeruginosa* isolates, related to saturation diving in the Norwegian sector of the North Sea, was selected on the basis of their PFGE genotypes [2–5]. The isolates comprised 28 genotypes with different origins, persistence and frequencies of occurrence in infections. Additional *P. aeruginosa* isolates from the North Sea, hospitals and the general community, as well as reference strains for *P. aeruginosa*, other *Pseudomonas* spp. and other bacterial genera, were also included in the study (Table 1). Sampling, cultivation and biotyping of the

**Table 1.** Bacterial isolates used in the study

| | ***Pseudomonas aeruginosa* genotypes**[a] | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Isolates** | **AD** | **AE** | **AL** | **AU** | **BM** | **D** | **E** | **G** | **N** | **P** | **TP1** | **TP2** | **TP4** | **TP5** | **TP12** | **TP27** | **Others**[b] | **NT** | **No. of isolates** |
| **Diving** | | | | | | | | | | | | | | | | | | | |
| Infections[c] | 5 | 1 | | 2 | 2 | 6 | 4 | 1 | 1 | 5 | | 1 | | | 1 | | 4 | 16 | 49 |
| Environment[d] | 4 | 1 | 2 | 1 | | 9 | 9 | 3 | 1 | | 2 | 3 | 2 | 2 | 1 | 5 | 3 | 3 | 51 |
| **Hospital** | | | | | | | | | | | | | | | | | | | |
| Infections | | | | | | | | | | | | | | | | | | | |
| Sepsis | Strains 1–9 | | | | **rDN, rEN, rDS, rDO, rEO, rEP, rEQ, rEM, rDH** | | | | | | | | | | | | | | 9 |
| Ear | Strains R1-R3 | | | | **rBB, rBC, rBG** | | | | | | | | | | | | | | 3 |
| Environment | Strain R4 | | | | **rCE** | | | | | | | | | | | | | | 1 |
| | 'Snøgg'[e] | | | | **xCCV** | | | | | | | | | | | | | | 1 |
| **Environment** | | | | | | | | | | | | | | | | | | | |
| Freshwater onshore | Strains K1, K2 | | | | **kCCZ, kDaA** | | | | | | | | | | | | | | 2 |
| **Reference strains** | | | | | | | | | | | | | | | | | | | |
| CCUG 551 T | | | | | **ER** | | | | | | | | | | | | | | |
| ATCC 27853 | | | | | **BBU** | | | | | | | | | | | | | | |
| PAO 1 | | | | | **aCB** | | | | | | | | | | | | | | |
| PA 103 | | | | | **aCC** | | | | | | | | | | | | | | |
| PAK | | | | | **aCA** | | | | | | | | | | | | | | |
| 388 | | | | | **aCD** | | | | | | | | | | | | | | |
| PAKS 1 | | | | | **BBD** | | | | | | | | | | | | | | |

**Non-*Pseudomonas aeruginosa***

| | | | |
|---|---|---|---|
| *Pseudomonas mendocina* | CCUG 1781 T | *Sphingomonas paucimobilis* | CCUG 6518 T |
| *Pseudomonas stutzeri* | CCUG 11256 T | *Brevundimonas diminuta* | CCUG 1427 T |
| *Pseudomonas alcaligenes* | CCUG 1425 A T | *Acinetobacter lwoffii* | CCUG 33984 T |
| *Pseudomonas fluorescens* | CCUG 1253 T | *Acinetobacter baumannii* | Own isolate |
| *Serratia fonticola* | CCUG 14186 T | *Escherichia coli* | Own isolate |
| *Serratia marcescens* | CCUG 1647 T | *Proteus mirabilis* | Own isolate |
| *Stenotrophomonas maltophilia* | CCUG 5866 T | *Klebsiella pneumoniae* | Own isolate |
| *Comamonas aquatica* | CCUG 15845 T | | |

T, type strain; CCUG, culture collection of University of Gothenburg; ATCC, American type culture collection; NT, non-typeable by PFGE (five RAPD-PCR genotypes).
[a]Designation according to PFGE profiles.
[b]Single genotypes: A, AY, J, TP13, TP28, V, AAY.
[c]Skin infections: external otitis, folliculitis, skin abscess, mostly localised in the face and neck areas.
[d]Isolates from freshwater, seawater, chamber interiors and divers' personal equipment.
[e]'Snøgg', a strain from a large hospital outbreak of infection in Norway.
PFGE, pulsed-field gel electrophoresis; RAPD, random amplified polymorphic DNA.