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Detection and Molecular Identification of Persistent Water Vessel Colonizing Bacteria in a Table Water Factory in Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Authors ON and MIN designed the study. Author ON performed the experiments and statistical analysis. Authors ON and MIN managed sample collection and author ON wrote the draft manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To establish organisms persistent in a table water production facility and to determine the points they entered in order to eliminate any risk of having pathogens in a water production system.
Study Design: Analytic observational studies
Place and Duration of Study: Bottling facility in Nigeria and University of Nottingham, United Kingdom. Study was between August 2011 and Sept 2013.
Methodology: Sample control points were subjected to counts of bacteria, yeasts, and mold using membrane filtration to trace the source of increased bacteria counts in a table water production factory. Organisms were identified by 16S rRNA sequencing and biofilm formation was assessed

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with micro titer dish biofilm formation analysis.
Results: Total bacteria and *Pseudomonas* Spp. count were highest in the carbon filter and 5% (v/v) chlorine used for disinfection was found to be effective against planktonic cells of 18 hour cultures of *Pseudomonas* Spp. isolated from the carbon filter tank. No yeasts and mold were detected and after a sand blasting exercise to clean affected tanks, total bacteria counts on tryptone glucose extract medium decreased to less than factory allowable limits of 25 cfu per 100 ml of water with no further growth of *Pseudomonas* Spp. on centrimide medium. Other bacteria that emerged after the sand blasting exercise were regarded as persistent but had less biofilm forming ability (p=.02) when compared with *Pseudomonas aeruginosa* PAO1. The persistent bacteria identified after sequencing were *Aeromonas hydrophila* and *Serratia proteamaculans.*
Conclusion: Apart from *Pseudomonas* Spp., other bacteria can persist in the water tanks of a water bottling facility and routine checks may fail to detect an underlying problem until it becomes obvious. Prompt corrective action ensures that public safety is not compromised. This was the first time *Aeromonas hydrophila* and *Serratia proteamaculans* were identified from the water tanks in the bottling facility.

Keywords: Persistence; water vessel; biofilms; Aeromonas hydrophila; Serratia proteamaculans; Pseudomonas Spp.

1. INTRODUCTION

Water processing facilities sometimes suffer microbial contamination which often leads to biofouling [1]. Water produced for public consumption is expected to be unmodified and the vessels, plumbing system and other equipment associated with production should be free of any defects that could lead to the contamination of the product. It is known that long-term storage of water can influence water quality and increase the number of microbial cells associated with biofilms on the interior surfaces of water storage tanks [2] and pipe works [3].

Virtually all bacterial species are known to have the ability to form biofilms and biofilms are defined as structured ecosystems in which microbes are attached to surfaces and embedded in a matrix composed of polysaccharides, eDNA, and proteins [4]. As reported previously [5], microbial biofilm colonies can form a smart material capable of responding to external threats dependent on their size and internal state and the microbial community accordingly switches between passive, protective, or attack modes of action. Also, microbial resistance to disinfection may lead to substantial economic losses [6].

Biofilms have been reported to be products of adhesion and growth of micro-organisms on surfaces which may cause bacterial contamination [7]. It has been explained that the ability of many bacteria to adhere to surfaces and form biofilms can create a persistent source of contamination [8] while another report pointed out that biofilms have probably existed since prehistoric times and evolved as part of the ability of microbes to cope with the harsh environmental extremes of earth present at that time [9].

The presence of bacteria containing pathogens in vessels of drinking water or water production lines can have serious implication to public health because they reduce the effectiveness of disinfecting chemicals which allows microorganisms to proliferate and cause bad flavours to drinking water [10,11]. The attachment of bacteria and subsequent development of biofilms is a potential source of contamination of finished products and can also cause microbial induced corrosion of water treatment vessels [12]. Bacteria has been implicated in the corrosion of different metals used in the food industry e.g. carbon steel [13], stainless steel [14], aluminum alloy [15] copper [16,17] and iron [18].

It has been noted [19] that control of microbial growth in drinking water distribution systems, often achieved through the addition of disinfectants (e.g. chlorine), is essential to limiting waterborne illness. Chlorine addition to water during the treatment process (reviewed in [20], will result in the formation of hypochlorous acid (HOCl) and hypochlorite ions (OCl-) and they can combine to make free chorine which has a high oxidation potential. The report also highlighted the leading advantage of chlorine because it is known to be inexpensive, effective against bacteria and viruses but the

disadvantage is that it can be toxic to humans at concentrations of up to 1000 ppm and it cannot inactivate all microbes and protozoan cysts.

Any water producing facility would not want biofouling because it can affect the company's profitability and cause possible product recalls, consumer complaints, law suits, and bad press or even out right closure of the company by regulatory agencies. Several man hours can be lost when infrastructure is dismantled in order to enable bio- tracing to eradicate biofilms and ensure that the production system can produce drinking water that meets specifications of international [21] and local regulatory agencies.

A table water producing company in south eastern Nigeria found increased bacteria count in sample from a critical control point (CCP) in their production process and a corrective remedial action plan was initiated to prevent bacteria from getting into the finished product which may put the consuming public at risk. Therefore, the aim of this work was to identify the persistent bacteria responsible for contamination and ascertain where it occurred in order to eliminate any associated risk of having pathogens in the water production system.

2. MATERIALS AND METHODS

2.1 Overview of Table Water Production Process

This work was carried out in a table water production factory in south eastern Nigeria and the flow of water as it is treated is shown in Fig. 1. To begin the table water production process in the factory, water (raw water) is pumped from a deep well into a holding tank and treated with chlorine. The water is then pumped to a chemical reaction tank and treated with various chemicals including iron and chlorine and then allowed to sediment. Sediment free water is pumped to a sand filter tank for another round of super chlorination and then transferred to a carbon filter for chlorine removal with the help of activated carbon present in the carbon filter tank. The chlorine free water exits the carbon filter to a polishing filter before it is transferred to the filling machine. Simultaneous filling and sterilization of the water with ozone (O_3) is carried out after which the water is filled into polyethylene terephthalate (PET) bottles and allowed to stand for 48 h to ensure dissociation of the ozone before the finished products are released into the market for sale.

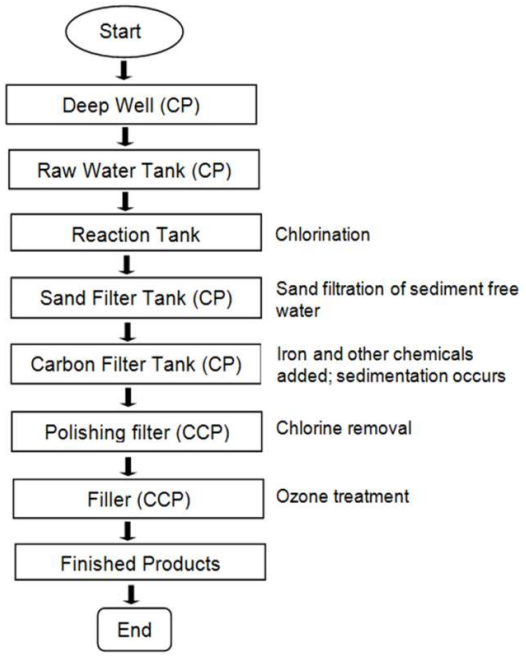

Fig. 1. Flow of water from a deep well until processed into drink-ready finished products. Each point is a control point (CP) except for polishing filter and filler which are critical control points (CCP)

The hazard analysis and critical control points (HACCP) system [22] is in operation in the plant and the sampling points are also known as control points (CPs) in the factory's microbiological monitoring program. Sampling points on the polishing filter tank and the bottle filling machine are regarded as critical control points (CCPs). Equipment is regularly inspected and disinfection is carried out with a clean in place (CIP) system by using 40 ppm chlorine water.

2.2 Sample Collection

Water samples (100 ml) for microbiological analysis were collected from sampling points fitted on various tanks in the production system with Whirl-Pak (Nasco, Wisconsin, USA) sampling bags and analyzed within 1 h after collection. Samples were collected from the following points namely, deep well, raw water tank, sand filter, reaction and carbon filter tanks, polishing filter and filler machine. Finished bottled water products were also taken from the bottling line for microbiological analysis. The group of organisms monitored in the factory was chosen by top management to satisfy local and

international regulatory requirements. Microbial count was reported as CFUs per 100 ml of water instead of standard CFUs per ml to satisfy the plants internal protocols.

2.3 Isolation and Count of Micro-organisms from Water Samples

Count of microorganisms was carried out using standard membrane filtration by placing 100 ml of water sample in a sterile multi-branched stainless steel manifold and filter holder system with different nutrient pad media sets (Sartorius, Göttingen, Germany) according to manufacturer's instructions. The nutrient pad media normally used in the factory for microbial analysis were specific for various groups of organisms. They included Tryptone Glucose Extract (TGE) media for total bacteria count, Tergitol triphenyltetrazolium chloride (TTTC) media pad for coliforms and enterobacteria, Centrimide (CT) nutrient pad for *Pseudomonas* Spp. and other non-faecal pathogenic bacteria and Schaufus Pottinger (SP) nutrient pad for yeasts and mould. The nutrient pad media composition is available on manufacturers website (https://www.sartorius.co.uk/). The multi-branched manifold and filter holder system has a tap in the base that allows vacuum to be turned on and off to draw off the water sample causing any microorganisms in the sample to be retained on a 0.45 µm membrane filter. The membrane filter was attached to a nutrient pad and incubated at 25℃ for 5 days for yeasts and mould and 37℃ for 24 h for bacteria. Incubation of cells on CT media was carried out at 42℃ for 48 hrs. Colonies that emerged were counted according to in house reporting system that expressed the counts as colony forming units per 100 ml of sample in order to comply with in local regulatory requirements. After abrasive treatment, swabs of the carbon filter interior surface were taken and broken off in 20 ml of 0.9% NaCl, sterilized by autoclaving in 30 ml universal bottles and then allowed to stand for 3 hours after which membrane filtration was carried out. Analysis was carried out with three replicates.

2.4 Test of Chlorine Disinfectant Efficacy

Microbial growth inhibition of isolated bacteria by chlorine used for disinfection was determined by method used in investigating the antibacterial effect of water-soluble tea extracts on foodborne pathogens grown in brain heart infusion growth medium [23]. The only modification was the use of membrane filtration for counting instead of spiral plating. Single colonies taken from TGE and CT media were used for the study. A loopful of organism of interest was inoculated in 10 ml of nutrient broth and grown at 37℃ with shaking for 18 h after which 1 ml of cells were introduced in 200 ml nutrient broth infusion with 5, 10, 15, 20, or 30% (wt./Vol) chlorine, a broth without chlorine served as a control. Incubation was carried out for 24 h at 37℃ after which membrane filtration of the nutrient broth infusion was performed using TGE and CT nutrient pad media. Incubation for samples on TGE pad media was for 24 h at 37℃ whereas CT was incubated at 42℃. Three independent cultures of the single colonies picked representing unknown species were analyzed.

2.5 Molecular Identification of Persistent Organisms

Swabs (Amies, Medical Wire, Wiltshire, UK) suitable for transport at ambient or refrigerated temperatures were used to pick three single colonies from TGE medium plates and sent to the University of Nottingham, United Kingdom for molecular identification. The swab samples were reconstituted on *Pseudomonas* agar base (Oxoid CM0559, Basingstoke, UK) with the normal supplement (Oxoid SR103) according to manufacturer's instructions after which sub-culturing was carried out on nutrient media. After standard Gram stain, oxidase and catalase tests were performed, duplicate samples of the three strains were stored in -85℃ for reference purposes.

Genomic DNA was extracted from cells grown overnight at 37℃ in nutrient broth using previous methods [24] following which PCR amplification of the V3 region of the 16S rDNA was carried out with the primers (Forward - CCTACGGGAGGCAGCAG; Reverse - ATTACCGCGGCTGCTCG) reported previously [25]. Initial denaturation was carried out for 5 min at 94℃ followed by annealing at 66℃, for 10 cycles with temperature decrease of 1℃ every cycle and then 20 cycles of annealing at 56℃. Extension for each cycle was performed at 72℃ for 3 min, while the final extension was carried out at 72℃ for 10 min after which the fragments were visualized on 2% agarose gel. The PCR fragments amplified (approx. 203 bp) were cut out from the gel and cleaned with gel purification kit (Qiagen, Hilden, Germany) and sequenced (Eurofin, Ebersberg, Germany). Sequences generated were subjected to a basic local

alignment search tool (Blast) activity on the NCBI data base(http://blast.ncbi.nlm.nih.gov/Blast.cgi) after which they were identified based on sequences of closest relatives. The sequences were submitted to European Molecular Biology Laboratory (EMBL) for curation and accession numbers and then deposited in the European nucleotide archives (ENA).

2.6 Assessment of Biofilm Formation

Biofilm formation was carried out using micro titer dish biofilm formation assay [26] with slight modification. Instead of using Luria broth, cells of three different colonies isolated from TGE and a laboratory standard strain of *Pseudomonas aeruginosa* PAO1 obtained from the University of Nottingham, United Kingdom culture collection were grown in 5 ml of nutrient broth at 37℃ for 18 h. The cultures in nutrient broth were diluted 1:100 after which 200 µl were placed in 96 well microtiter plates (Fisher Scientific, UK) and grown for 24 h. Staining was carried out with crystal violet (Fisher Scientific, UK) and quantification of the biofilm was performed in a plate reader (Victor^{TM}) by measuring absorbance of solubilized crystal violet at 600 nm instead of 550 nm. Three independent batches of cells were assessed.

2.7 Statistics

Analysis of variance was performed with a *p* value set at .05 using the data analysis ToolPak of Microsoft™ Excel 2010.

3. RESULTS AND DISCUSSION

3.1 Microbial Growth and Counts

An initial microbial analysis carried out at the beginning of the study showed that out of the 4 nutrient pad sets that were used, only total colony count (TGE) and *Pseudomonas* spp. (CT) media had growth (Table 1).

In fact, no growth was observed from TTTC and SP media throughout the study which indicates that coliforms, yeasts and mold were absent in the water production system at the time of analysis. Also no growth was detected from the sand filter sampling point and finished products analyzed for the entire duration of the study which suggests that disinfection with high chlorine concentration and ozone for finished products were effective in stopping most of the microorganisms from proliferating. Furthermore, the lack of growth in TTTC medium indicates lack of fecal contamination while the lack of growth in SP media may likely be because plain water samples without any supplement like glucose is unfavorable for yeast growth. Media containing centrimide allows good growth of *Pseudomonas* Spp. [27] and observed growth on CT media at 42℃ indicated that the organism was likely to be *P. aeruginosa* because the organism grows at 42℃ whereas most other *Pseudomonas* Spp. will not grow at 42℃ [28]. It was concluded that *P. aeruginosa* was probably the dominant organism that was responsible for fouling the carbon and polishing filters. Lack of growth in the sand filter is likely due to super chlorination of the tank and the bacterial free finished water products show that the increase in counts in the system was not able to overwhelm the sterilizing effect of ozone treatment during bottling.

Bacteria count was highest in the carbon and polishing filter and their counts was over the factory limits of 25 colonies per 100 ml of water whereas counts of the deep well, raw water and reaction tanks and filler machine where within the allowable limits (Fig. 2a). It was concluded that the carbon filter was the source of contamination since water going into the carbon filter from the sand filter had zero growth.

Table 1. Occurrence of microorganisms on TGE, TTTC, CT and SP media (+ = present/ - = absent)

Sample point	Before sand blasting				After sand blasting			
	TGE	TTTC	CT	SP	TGE	TTTC	CT	SP
Deep well	+	-	-	-	+	-	-	-
Raw water tank	+	-	-	-	+	-	-	-
Reaction tank	+	-	-	-	+	-	-	-
Sand filter	-	-	-	-	-	-	-	-
Carbon filter	+	-	+	-	+	-	-	-
Polishing filter	+	-	+	-	-	-	-	-
Filler	-	-	-	-	-	-	-	-
Finished products	-	-	-	-	-	-	-	-

The surface of the organisms may have contributed to attachment of bacteria on the carbon and polishing filters and it has been shown by others [29] who performed physicochemical analysis on cell surface of *P. aeruginosa* and found that the organism showed higher hydrophobicity and surface charges along with the capability of producing extracellular polymeric substances (EPS). It is possible that these features led to high adhesive force on the surface of the carbon filter. In order to reduce the high bacteria counts, the chlorine concentration used for CIP disinfection was increased from 40 ppm to 80 ppm. After increase in chlorine concentration membrane filtration analysis carried out showed a decrease in bacteria count (Fig. 2b) for the carbon filter and polishing filter but the counts (Fig. 2c) taken a week later showed an increase despite the higher chlorine concentration used.

It is known that *Pseudomonas* will form biofilms under any condition that allows growth [30] and biofilm formation by *Pseudomonas* is facilitated by flagella and twitching motility [31] after which cells form cell-to-surface and cell-to-cell contacts resulting in the formation of micro colonies. It has been reported [32] that *Pseudomonas* is capable of surviving and proliferating in water distribution systems and another report [33] highlighted that water output from a complex network of interconnected dental unit waterlines can be contaminated by high densities of microorganisms, principally Gram-negative environmental bacteria including *P. aeruginosa*. From a health perspective, drinking-water biofilms can act as a reservoir for *P. aeruginosa* and is considered as a source of contamination in water distribution systems [34]. Other species of *Pseudomnas* like *P. fluorescens* is also an excellent biofilm former [35].

Mixed growth on TGE was expected because it is a non-selective media and indicates that other organisms may have contributed to the resistance of *Pseudomonas Spp.* to disinfection chemical used because it has been suggested that the diversity of isolates from drinking water can facilitate resistance of biofilms to disinfection [36]. Again, metabiotic generation of substrates for *Pseudomonas spp*. by other organisms may have contributed to *Pseudomonas spp.* domination [37].

When it was established that the carbon filter was the root cause of the higher counts experienced in the factory, an abrasive blasting (sandblasting) of the tank was arranged by the bottling plant company and carried out on the carbon filter tank while the polishing filter was stripped, scrubbed manually and changed the filtration cartridges. The sand blasting was outsourced and it involved forcibly propelling a stream of sand against the tank's internal surface under high pressure to remove biofilm and any other surface contaminants before recoating the surface with epoxy paint.

After the sand blasting exercise, microbial analysis carried out showed growth only on TGE medium and no growth on the CT or other media (Table 1). Counts of colonies on TGE medium showed that there was reduction in counts from the carbon and polishing filters (<6 cfu per 100 ml, data not shown) with no increase a week after. Counts were within the plant's target of less than 25 cfu per 100 ml and also the lack of growth of *Pseudomonas* Spp. on CT medium a week after the sand blasting exercise is most likely due to the fact that biofilms were removed. It has been reported [38] that *Pseudomonas* Spp. is not often found in drinking water and that its occurrence in drinking water is probably related more to its ability to colonize biofilms in plumbing fixtures. Biofilms have been shown to form when cells first attach to a surface and then start to proliferate, before finally the biofilm reaches maturity [39] and become less likely to be removed. Therefore, it is possible that the biofilms in this study failed to reach maturity and failed to become irreversibly attached to the carbon filter surface which made it possible for the biofilms to be removed by the sandblasting treatment.

3.2 Effect of Using Chlorine on Dominant Bacteria

Chlorine inhibits microorganisms by cell membrane damage which allows chlorine entry into the cell to disrupt cell respiration and DNA activity necessary for cell survival [20]. The investigation started when it was observed that there were increases in the total bacteria count when counts from all points were summed up (none in the product). In order to verify that the concentration of chlorine used for equipment disinfection was still efficient, tests were carried out with various chlorine concentrations and it was found that there was growth too numerous to count in control test tube samples without chlorine while samples containing chlorine showed no growth on TGE or CT media in all chlorine concentrations (5-30% w/v) tested. This confirmed that the chlorine concentration used

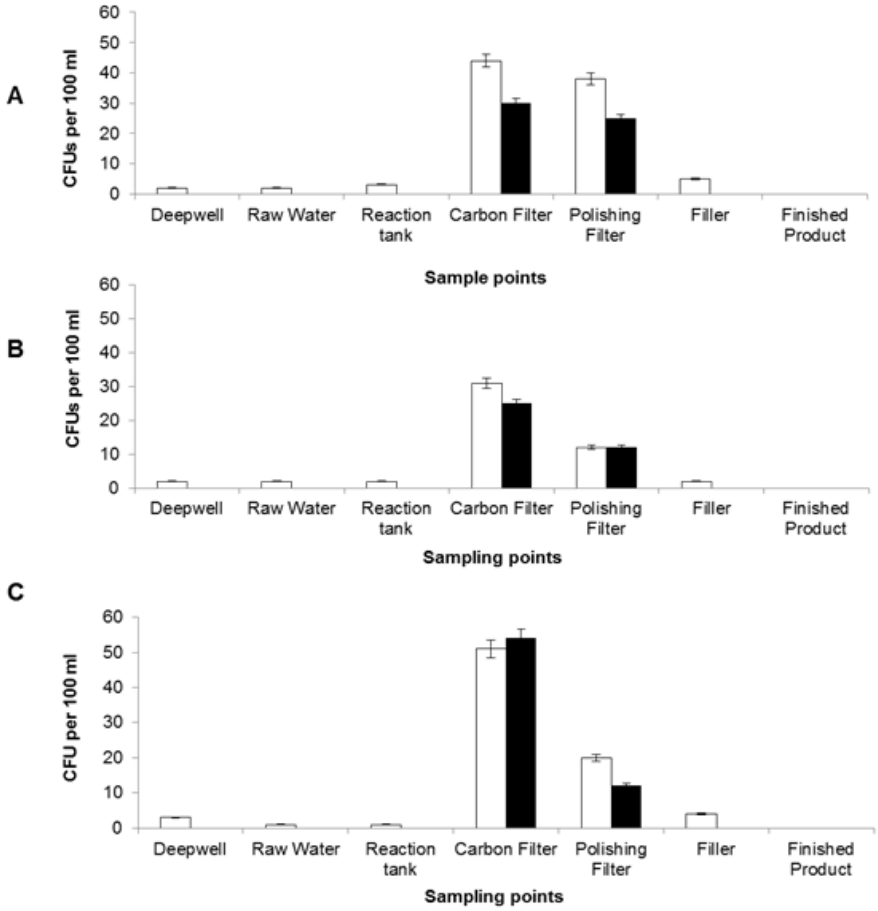

Fig. 2. Initial count (A) of sampling points (control points) with growth on TGE (□) and CT (■). Counts were taken after increase of disinfection chemicals from 40-80 ppm of chlorine (B) and a further count taken a week after increase in disinfection chemicals (C)

for equipment sanitation in the factory was efficient against planktonic cells isolated from the carbon filter tank. These results increased suspicion that the problem could be cells attached to the tank. Microorganisms can influence corrosion of metals [40] and aerobic corrosion of steel can be influenced by *Pseudomonas* [14], hence it is possible that attachment of bacteria to the carbon filter surface facilitated an increase in chlorine disinfection resistance [41].

3.3 Molecular Identification of Persistent Organisms

In order to establish the organisms that persisted after the sand blasting of carbon filter exercise, the tank was swabbed and plated on TGE and CT media. Single colonies were picked directly from plates of TGE to identify few persistent bacteria that emerged following membrane filtration analysis. Single colonies were taken and reconstituted on *Pseudomonas* agar base with *Pseudomonas* CFC supplement SR0103 used for growth of any *Pseudomonas* spp. instead of supplement SR0102 which is recommended for *P. aeruginosa*. As shown in the manual, use of supplement SR0103 with lower centrimide content (5mg per vial) rather than supplement SR0102 with higher centrimide content (100 mg per vial) is advised if the colonies of interest are different species of *Pseudomonas*. Since no growth was observed on the CT media in the factory after abrasive treatment, the vial with lower centrimide content was used to prepare the media to ensure that other species of *Pseudomonas* are covered. The colonies that emerged after incubation were intact, raised and all colonies were Gram negative and catalase positive. Two colonies were creamy to yellowish

and oxidase negative while the other colony was slightly brownish and oxidase positive.

The PCR reaction yielded fragments of approximately 200 bp (Fig. 3) and sequencing of the fragments identified the 2 yellowish colonies to be *Serratia proteamaculans* and the brownish one to be *Aeromonas hydrophila*.

The sequences were deposited with EMBL and given accession numbers HG328350 to HG328352. Closest match of identified persistent organisms, their percentage homology from the nucleotide sequence search carried out, accession numbers of closest relative and assigned accession numbers from ENA are shown in Table 2.

Since *S. proteamaculans* is a member of the enterobacteriaceae family [42], the unexpected appearance on *Pseudomonas* media used may be because the media has the deficiency of supporting growth of some undesirable enterobacteriaceae as stated in the manufactuer's manual and confirmed by others [43]. Growing of *Aeromonas* on *Pseudomonas* media has been demonstrated when glutamate starch phenol red agar was used [44] to grow *Pseudomonas and Aeromonas* Spp. isolated from fish simultaneously on the same plate.

The organism *A. hydrophila* is known to occur in water and in a previous review [45], it was highlighted that the genus *Aeromonas* can be isolated from rivers, lakes, ponds, seawater (estuaries), drinking water, groundwater, wastewater, and sewage in various stages of treatment. Also, a genus-specific real-time quantitative PCR assay have found *Aeromonas* isolates from both raw and treated water samples and the qPCR analysis indicated presence of a considerable non-culturable population of *Aeromonas* in drinking water samples [46]. Another investigation of naturally developed biofilms in a drinking water treatment plant found *A. hydrophila* in the active microbial consortia in the biofilm assessed [47]. Furthermore, the organism can withstand prolonged starvation and shift from the normal rod shaped cells to spherical forms to survive for long periods when inoculated in mineral and drinking chlorinated water [48], so it is possible the *A. hydrophila* identified in this study exhibited similar survival strategy to grow and persist in the carbon filter vessel after sandblasting.

It has been reported that *Serratia* Spp. can thrive in a diverse number of environments that include water and produce various secondary metabolites that directly contribute to their ability to resist attack, respond appropriately to environmental conditions, and out-compete other microorganisms [49]. Previous reports show that *S. proteamaculans* is a secondary invader of infections [50] that produces the enzyme metalloprotease which has high activities at wide pH and temperature ranges and depends on the presence of metal ions such as calcium and zinc for its enzymatic activities [51]. It is possible the occurrence of this organism in the carbon filter was preceded by occurrence of other organisms.

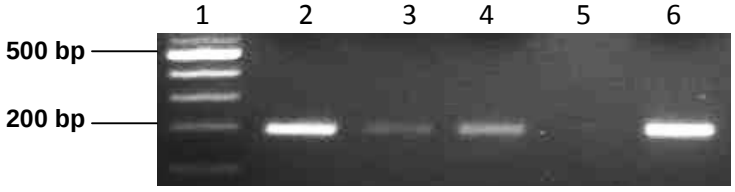

Fig. 3. PCR amplification of the hyper variable V3 region of the 16S rDNA Lane 1 =100bp ladder; Lane 2 = *Aeromonas hydrophila*; Lanes 3 and 4 = *Serratia proteamaculans*; Lane 5 = negative control; Lane 6 = positive control - *Pseudomonas aeruginosa* PAO1

Table 2. Strains identification of persistent strains by 16S rRNA sequencing

Strain	Closest relative	% identity score	NCBI accession No	Assigned ENA accession No
P2	*S. proteamaculans*	99	KC178580.1	HG328350
P3	*A. hydrophila*	99	KC202273.1	HG328351
P4	*S. proteamaculans*	99	KC178580.1	HG328352

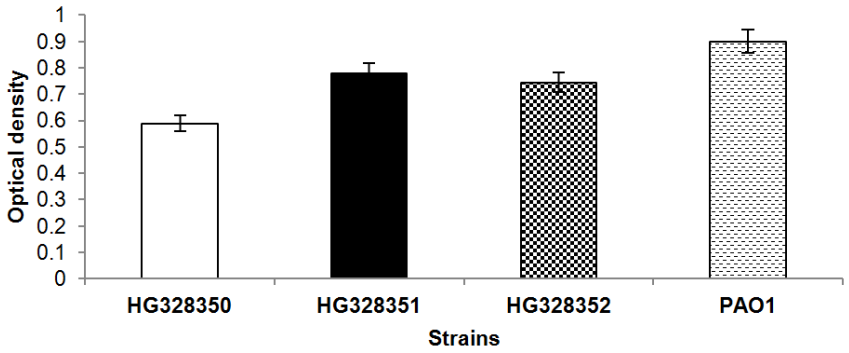

Fig. 4. Biofilm forming ability of sequenced strains compared with *Pseudomonas aeruginosa* PAO1

3.4 Biofilm Assay

In other to establish if the sequenced strains have biofilm forming ability greater than known heavy biofilm producers like *Pseudomonas*, the two strains of *S. proteamaculans* and strain of *A. hydrophila* isolated from the carbon filter after sand blasting activity were compared to a standard strain *P. aeruginosa* PAO1. Results (Fig. 4 above) show that the biofilm formed by the standard *P. aeruginosa* PAO1 is significantly (p=.02) more than strains isolated from the carbon filter.

The biofilm forming abilities of *P. aeruginosa* and its life style in biofilms is well known [52] but the implication of *S. proteamaculans* and *A. hydrophila* as biofilm formers in food industry and hospitals is not widely reported. However, it has been established recently that *S. proteamaculans* outcompeted *Listeria monocytogenes* in a dual species biofilm on the surface of food grade stainless steel by exhibiting similar desiccation survival strategy and forming separate micro colonies [53]. Also an invitro biofilm assessment [54] of 45 strains of *A. hydrophila* isolated from rivers showed that there were weak, moderate and strong biofilm producers among strains isolated.

4. CONCLUSION

The detection of *Pseudomonas* Spp. and subsequent molecular identification of *A. hydrophila* and *S. proteamaculans* highlights the succession that took place in the water treatment plant and clearly gave the factory management new information that *Pseudomonas* Spp. is not the only organism that is important in carbon filter fouling. The biofilms formed by *Pseudomonas* Spp. was not permanent or irreversible. Growth of *A. hydrophila* and *S. proteamaculans* on the carbon filter interior surface and proliferation on selective media for *Pseudomonas* indicates their persistence and ability to grow in a wide range of growth conditions. The study showed the importance of early detection warning systems to prevent contamination of the final product in a production process and highlights the importance of CPs which can serve as an early warning signal station in a water production facility. The corrective action taken prevented microorganisms in the biofilms from contaminating finished products released for sale (drink-ready water) and this indicates that the hazard analysis and critical control points (HACCP) program in place at the factory was efficient and the water factory was in charge of their environment. The study also shows the need for implementation of more monitoring tools that would enable bio-tracing and early warning systems in a water processing system.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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