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Research Article

Evaluation of the biofilm's microflora of drinking water pipes in Sidi-Bel-Abbes city, Algeria

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Abstract:

The development of biofilms on the inner-pipe surfaces of drinking water constitutes one of the major microbial problems, which contributes to the deterioration of water quality and entails potential health risks for consumers. Data about the biofilm's microflora of real chlorinated drinking water distribution systems (DWDS) were collected, on the ground in Sidi-Bel-Abbes (SBA) city, Algeria. A set of analyses were conducted to evaluate, to identify, and to determine the prevalence of bacterial organisms isolated from biofilms. Thirty biofilm samples were collected from the internal walls of the drinking water pipes. The bacterial abundance and the composition of biofilm's communities were analysed by heterotrophic plate counts and identified by Gram's reaction, cultural features and biochemical characterisation. Despite the presence of free residual chlorine in the drinking water, biofilm density varied between $2.4x10^5$ and $9.8x10^8$ colony forming unit/cm^2 (CFU/cm^2). Simultaneously, a higher diversity of the bacterial communities was detected. They were listed in two bacterial groups. The predominant group was Gram-negative bacilli, with a rate of 67.86%, including *Pseudomonas, Escherichia, Klebsiella, Enterobacter, Citrobacter, Yersinia,* in which *Pseudomonas aeruginosa* was at the top rate. However Gram-positive cocci group rate was 32.14%; including two genera *Staphylococcus and Enterococcus,* in which *Staphylococcus aureus and* coagulase-negative *Staphylococcus* represented the majority of isolated strains. Therefore drinking water biofilms constitute a reservoir of opportunistic pathogens which can be harmful to human health. For this reason, it is recommended to optimise the water treatment sectors in Algeria so as to limit biofilm's development, water quality degradation, and protect public health.

Keywords: Biofilm, microflora, diversity, drinking water distribution systems, *Pseudomoanas*.

1. INTRODUCTION

The distribution networks of drinking water are constantly exposed to a flow of microorganisms. This flow can happen by escaping the treatment and disinfection processes (breakthrough) or by intrusion, due to external contamination events in different steps of water treatment, storage and transportation: cross-connections, backflow events, pipe breaks, etc [1]. Despite the fact that drinking water distribution systems (DWDS) are extreme environments with oligotrophic conditions where a disinfectant residual is commonly maintained, the majority of these microorganisms are able to survive, in particular by attaching to the internal surfaces of pipes forming biofilms [2].

Biofilms are bacterial communities embedded in a matrix of extracellular polymeric substances (EPS), which gives them the opportunity to resist destruction by environmental stress and biocides [3]. Different factors within DWDS might influence the development of biofilm: microbial quality of intake water, biodegradable organic matter, residual disinfectants, environmental factors, hydrodynamics, water residence time and type of pipe materials [4].

Biofilm mobilisation in real systems induces residual disinfectants depletion [1] and could cause water quality failures, due to the detachment of cells and (in) organics concentrated in the EPS [5]. This could permit the survival and proliferation of a variety of opportunistic pathogens such as *Pseudomonas, Aeromonas, Klebsiella, Mycobacter, Escherichia coli, Helicobacter, Salmonella* and *Legionella*, which are responsible for several waterborne diseases

including gastroenteritis, diarrhoea, cholera, typhoid fever, meningitis, dysentery, hepatitis, legionellosis, pulmonary infections, giardiasis, etc [6].

This paper represents the first attempt to investigate the biofilm's microflora of the real drinking water distribution systems in Sidi-Bel-Abbes (SBA) city (north-west of Algeria). Hence, the aim of this study was to evaluate, to identify, and to determine the prevalence of bacterial organisms isolated from biofilms. Therefore, the knowledge of the biofilms' microflora is essential to improve control and management strategies in DWDS in SBA.

2. MATERIAL AND METHODS

2.1. Sampling sites

This study was conducted during a period of one year at the city of SBA (north-west of Algeria). Thirty (30) biofilm's samples were collected from various points over the entire tertiary distribution network, taken from the internal walls of pipes made of polyvinyl chloride (PVC).

2.2. Collection of samples

The collection of biofilms was done by friction, using sterile swabs, from the internal walls of the drinking water pipes. It was done directly on site. The 1st swab, of a surface of 1 cm^2, was used to count the total aerobic mesophilic flora and put in a tube containing sterile physiological water. The 2nd swab, used to isolate the microorganisms, was put in a tube of nutrient broth.

2.3. Storage and transport of samples

The collected samples were stored in the cooler under a temperature between 4°C to 6°C, and sent to the laboratory immediately.

2.4. Preparation of the samples

The tube of sterile physiological water, containing the swab, was vortexed for one minute to take off the bacteria and disperse the samples [7, 8] from which serial decimal dilutions were carried out.

2.5. Enumeration of the biofilm's microflora

From the different dilutions, the Petri plates were inoculated using the method of incorporation in nutrient agar and incubated for 72 hours at 30°C. The results were expressed in Colony Forming Unit (CFU) per cm^2 of surface area [8].

2.6. Isolation and identification of the biofilm's microflora

The bacteria were isolated and identified according to standard methods.

2.6.1. Enrichment

The inoculated nutrient broth was incubated for 20±4 hours at 36±2°C.

2.6.2. Isolation of bacteria

The collected microorganisms from the enrichment broth were then put in culture on two selective media: Mac Conkey for the isolation of Gram-negative bacilli; Chapman for the isolation of Gram-positive cocci. Then, they were incubated at 36±2°C during 20±4 to 44±4 hours.

2.6.3. Purification of isolates

After the incubation of media, isolates in pure culture were realised from the different colonies of the original Petri plates to obtain pure strains. These strains were cultured on nutrient agar for 20±4 hours at 36±2 ° C.

2.6.4. Identification of isolates

The identification of purified strains was through: macroscopic and microscopic examinations (fresh state and Gram staining), respiratory type, search for enzymes (catalase, oxidase, coagulase) and biochemical analyses using kits API 20E, API 20Strep (BioMérieux, France).

3. RESULTS

3.1. Quantitative evaluation of the biofilms' microflora

The results obtained indicate that all the pipes have a fixed biomass, distributed in a heterogeneous manner, and countable by the agar incorporation method; despite the disinfection of the distributed water; it varies from $2.4x10^5$ to $9.8x10^8$ CFU/cm^2.

3.2. Qualitative evaluation of the biofilms' microflora

3.2.1. Identification of the biofilms' microflora

From the biofilm samples, and at the base of the biochemical identification, a total of 252 strains were isolated and identified such as the bacteria of eight (8) different genera. These include, in descending order of predominance: *Staphylococcus* 23.81%; *Pseudomonas 19.84%*; *Escherichia* 11.51%; *Klebsiella* 10.32%; *Enterobacter* 9.92%; *Citrobacter* 9.13%; *Enterococcus* 8.33% and finally *Yersinia* 7.14%, (Figure 1).

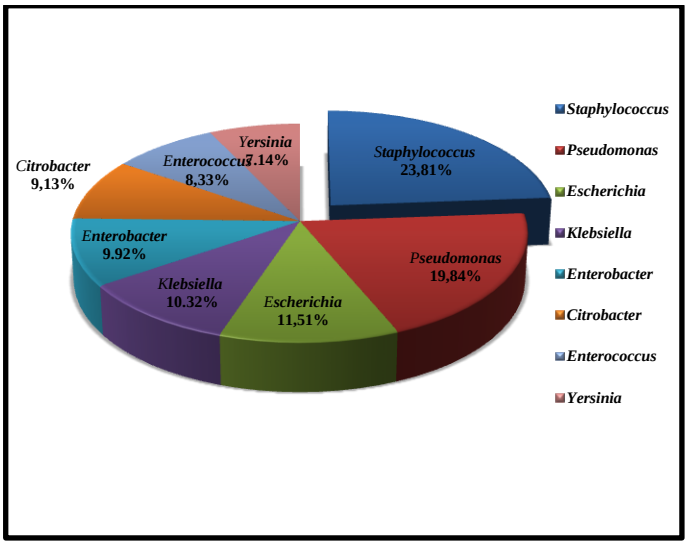

Figure 1: **Distribution rates of bacterial genera in the biofilms**

3.2.2. Distribution of bacterial groups in the biofilms

The microscopic examination (morphology and Gram type) of

the 252 strains isolated from drinking water distribution pipes, allowed us to list two (2) bacterial groups; these are predominantly Gram-negative bacilli, with a rate of 67.86% (171 strains/252); and Gram-positive cocci at 32.14% (81 strains/252), (Figure 2).

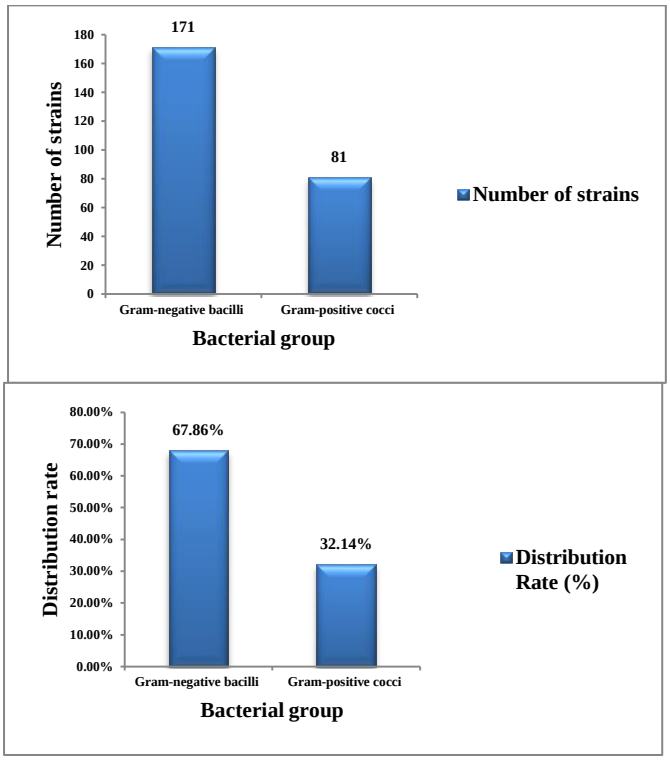

Figure 2: **Distribution of bacterial groups in the biofilms**

3.2.3.1. Distribution of Gram-negative bacilli in the biofilms

This study revealed that Gram-negative bacilli were the most frequently isolated bacteria in the drinking water pipes of SBA city. These include, in descending order of predominance, 11 bacterial species: *Pseudomonas aeruginosa* with the highest prevalence rate of 17.54% (*30* strains*/171) ; Citrobacter freundii* and *Escherichia coli* 13.45% (23/171) for each ; *Pseudomonas fluorescens* 11.70% (20/171) ; *Yersinia aldovae* 10.53% (18/171) ; *Enterobacter agglomerans* 8.19% (14/171) ; *Klebsiella pneumoniae* 7.60% (13/171) ; *Enterobacter amnigenus2* 6.43% (11/171) ; *Klebsiella pneumo.sp ozaenae* 4.09% (7/171) ; *Escherichia vulneris* and *Klebsiella oxytoca* with the same rate of distribution 3.51% (6/171), (Figure 3).

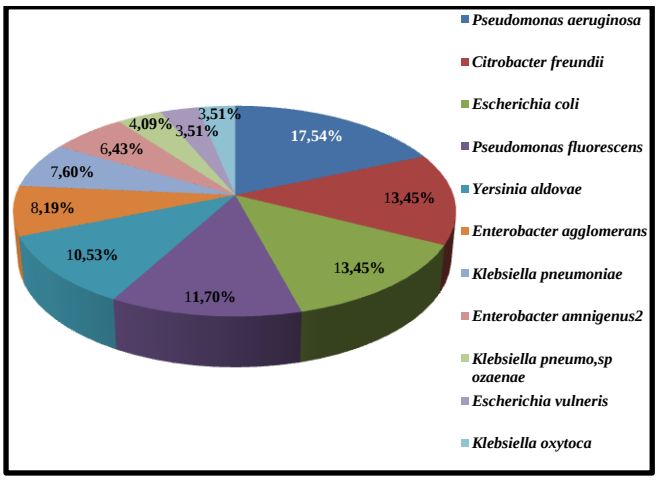

Figure 3: **Distribution rates of Gram-negative bacilli in the biofilms**

3.2.3.2. Distribution of Gram-positive cocci in the biofilms

The Gram-positive cocci include only three (3) different bacterial species, with a prevalence of *Staphylococcus aureus* and coagulase-negative *Staphylococcus* in the first position with 37.04% (30/81strains) for each, then *Enterococcus faecalis* 25.93% (21/81), (Figure 4).

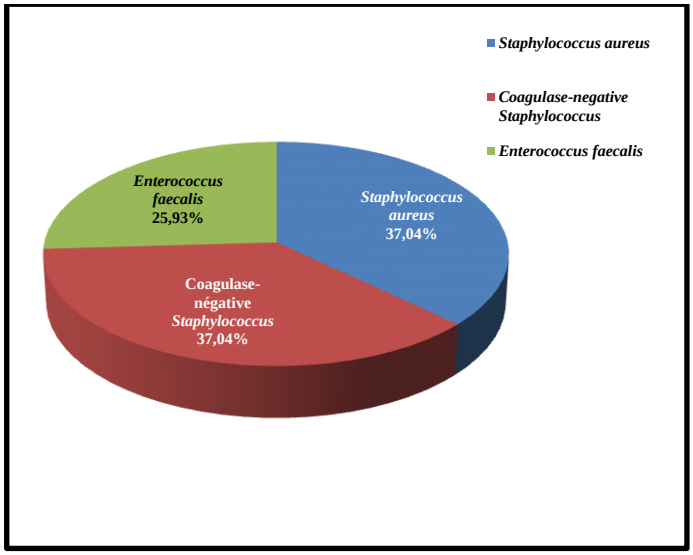

Figure 4: **Distribution rates of Gram-positive cocci in the biofilms**

4. DISCUSSION

The contribution of biofilms to the contamination of distributed water is a major public health issue. Hence, improving our knowledge about the microbiota that constitutes the biofilm of drinking water pipes is at the basis of understanding the phenomena related to the quality's degradation of the distributed water. For this reason, quantitative and qualitative analyses of the bacterial communities were carried out, within the biofilms grown in the internal walls of drinking water pipes.

In this study, the quantitative analysis of the biofilm's microflora revealed significant contamination of drinking water pipes' network from SBA city. The results of the enumeration of total mesophilic aerobic flora were between $2.4x10^5$ and $9.8x10^8$ CFU/cm^2. Moreover, September and al.[9] found values reaching 10^9 CFU/cm^2.

The quantitative variations of the fixed biomasses, on the internal surface of the pipes, were observed from one sampling point to another. These variations were explained by the heterogeneity in the distribution of the biofilms inside the network's supply in SBA, this might be due to different factors. First, the age of pipes sampled, Martiny and al.[10] observed that the biofilm developed from single cells through the formation of independent microcolonies reaching a thickness ranging from 14.1 to 30 µm, depending on the biofilm's age. At the end of three years, the microcolonies covered 76% of the surface. Second, the nutrient supply, Block and al.[11]calculate the time of biomass doubling fixed during few days to several tens of days; depending on the

nutrient status of the water. Third, the water residence time in the pipe and the flow velocity, Lehtola and al.[12] show that the acceleration of the flow velocity increases the growth of biofilms. Fourth, the external contaminations such as cases of cross-connections and water leakage, which are frequent in the distribution networks of SBA.

The present study showed colonisation of the internal surfaces in spite of the chlorination of the distribution networks. According to Paquin and al.[13], the action of chlorine vis-à-vis the biofilm is superficial; they use 3.40 mg/L of chlorine for a reduction in the bacterial density of the biofilm of 0.91 logs. In addition, DeBeer and al.[14] observe that the concentration of chlorine on the biofilm's surface is 20 to 30% of that in the water phase because of the existence of the boundary layer.

Our results revealed that biofilms' microflora is constituted mainly of bacteria found in sanitary control including indicators of fecal contamination[15]. In South Africa, a similar study on the biofilm composition of DWDS shows the presence of total coliforms, fecal coliforms, *Pseudomonas spp* and *Aeromonas spp*[16]. Moreover, another study in Nigeria shows in decreasing order of predominance the presence of *P. aeruginosa*, coliforms, *Salmonella typhi*, *E. coli*, *Aeromonas hydrophila*, *Streptococcus*, *Legionella pheumophila*[17].

We identified two different groups of bacteria with a predominance of Gram-negative bacilli. This was similar to those obtained by Hamieh and al.[18]. This group included genera like *Pseudomonas, Escherichia, Klebsiella, Enterobacter, Citrobacter, Yersinia*, and species like *P. aeruginosa, Citrobacter freundii, E. coli, P. fluorescens, Yersinia aldovae, Enterobacter agglomerans, Klebsiella pneumoniae, Enterobacter amnigenus2, Klebsiella pneumo.sp ozaenae, E. vulneris*, and *Klebsiella oxytoca*.

The predominance of *Pseudomonas* including *P. aeruginosa* and *P. fluorescens*, could be explained by the fact that it is able to produce high amount of extracellular polymeric compounds, which favor the formation of biofilms[19, 20] on various types of biotic and abiotic surfaces, such as the pipes of water[21]. Indeed, Bédard and al.[22] shows that chlorine causes mortality of the bacterium; however *P. aeruginosa* regain viability quickly after the depletion of free chlorine, while cultivability is recovered within 24 h. *Pseudomonas* can be good indicators of biofilm development risk in water networks[23].

A widespread contamination, with coliforms which are bacteria used as indicators of fecal contamination of drinking water[24]was detected. The genera were *Escherichia, Klebsiella, Enterobacter, Citrobacter,* and *Yersinia*. According to the study conducted by Volk and al.[25], this contamination could be explained through several factors such as temperature (above than 15°C), a content of biodegradable organic matter (greater than 0.15 mg/L), bacterial flora in water suspension (greater than 5.2 log), a residual chlorine (less than 0.10 mg/L). These germs are able to survive in the networks' distribution and to multiply within the biofilm [26]. In fact, Fass and al.[27] shows that during an experimental injection of a water distribution system, a few hours were sufficient for a strain of *E. coli* to colonise the biofilm. Whereas, a week later, only a few coliforms were still presents in the water phase of the network. Furthermore, the results of Kilb and al.[28] suggest that coliforms found in distributed water would come from the biofilm.

The group of the Gram-positive cocci was represented by two (2) genera: *Staphylococcus and Enterococcus.* Firstly, *Staphylococcus* was the predominant genus including *S. aureus* and coagulase-negative *Staphylococcus*, which are ubiquitous organisms. Indeed, higher percentages of *S. aureus* occurrence in chlorinated drinking water are reported worldwide. In addition, Staphylococci are well known for their ability to produce biofilm on different surfaces such as distribution pipelines, and can deteriorate the overall drinking water quality[29]. Similar species were detected by Hamieh and al.[18] in their study on a distribution network in Lebanon.

Secondly, the genus *Enterococcus* presented by *Enterococcus faecalis* is a sign of fecal contamination in distributed water. These species survive longer in water than coliforms and other enteric bacteria[30]. Indeed, to integrate with the biofilm, bacteria must first survive the treatment[31].

As a whole, we found that several bacteria isolated from distributed water were adhered and fixed on the inner walls of the drinking water pipes of SBA city. These potentially pathogenic bacteria were often involved in opportunistic or nosocomial infections, among them: *P. aeruginosa, S. aureus*, coagulase-negative *Staphylococcus*, *Enterobacteriaceae*, as well as indicator germs of pollution. According to Amazian and al.[32], a Mediterranean prevalence survey in 2010, shows that *P. aeruginosa* ranks third after *E. coli* and *S. aureus* in nosocomial infections. Joly and Reynaud[24] claim that enterobacteria were also found, for twenty years, in half of the nosocomial infections. The species responsible were mainly *E. coli* but also *Klebsiella, Enterobacter, Serratia, Proteus, Providencia*.

5. CONCLUSION

Contamination of the internal walls of drinking water pipes is a problematic that is treated for the first time in SBA, north-west of Algeria. This study is intended to draw the attention of public health authorities, caterers and water distributors in Algeria to intensify their efforts to monitor and control the quality of the Algerian drinking water supply systems.

Despite the chlorine disinfection of the distributed water, the results of this study revealed a high fixed biomass rate ($2.4x10^5$ at $9.8x10^8$ CFU/cm^2), as well as a large diversity of bacterial communities within the biofilms in the drinking water network. These biofilms perform great capacity of adaptation to ultra-oligotrophic conditions; degrade the quality of distributed water by the release of adherent bacteria, and cause serious infections.

The strategy of disinfection with chlorine is inadequate vis-à-vis the biofilms, that is why, preventive measures are effective to control this accumulation in distribution network. Therefore, it would be interesting to optimise the treatment sectors in Algeria, in order to minimise the organic matter and the microorganisms at the outlet of the water treatment plant.

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