Research Journal of Pharmaceutical, Biological and Chemical Sciences

(ISSN: 0975-8585)

RESEARCH ARTICLE

Detection Of Potential Pathogenic *Pseudomonas aeruginosa* In A Hospital Water System

Allan Demétrius Leite de Oliveira[1], Ulrich Vasconcelos[2]*, and Glícia Maria Torres Calazans[3].

[1]Centro Universitário Dr. Leão Sampaio, Av. Dr. Leão Sampaio s/n, Campus Saúde, CEP – 63040-005, Juazeiro do Norte-CE, Brasil.
[2]Centro de Biotecnologia, Universidade Federal da Paraíba – Campus I, R. Ipê Amarelo s/n, CEP – 58051-900, João Pessoa-PB, Brasil.
[3]Departamento de Antibióticos, Centro de Biociências, Universidade Federal de Pernambuco, Av. Prof. Moraes Rego, s/n, CEP – 50740-901, Recife-PE, Brasil.

ABSTRACT

Pseudomonas aeruginosa is commonly found in the hospital environment and poses a threat to patients because it is a very significant contaminant in pipes, equipment, cleaning materials, antiseptics, pharmaceuticals and water. This work reports the monitoring of *P. aeruginosa*, both total coliforms and thermotolerant coliforms, in water from a hospital for a period of sixteen weeks. Water samples were collected from the treatment plant (WTP), storage tank (ST) and taps in the adult ICU, neonatal ICU and hemodialysis unit. There was an increase in the number of *P. aeruginosa* and coliform bacteria in tap water of the adult and neonatal ICUs, compared to the WTP and ST. Twelve pyocyanin-producing *P. aeruginosa* specimens resistant to more than 80% of tested antibiotics were recovered. The results indicate the risk of health-care associated infections by opportunistic microbes found in water, as well as the need for stricter monitoring of water distributed in hospitals, and the inclusion of microbiological parameters in addition to those for the control of water for human consumption.

Keywords: hospital environment, water monitoring, healthcare-associated infection, pseudomonads.

https://doi.org/10.33887/rjpbcs/2021.12.4.18

Corresponding author

INTRODUCTION

Health-care associated infections (HCAIs) are the major cause of death and disease arising in hospital environments. These include both buildings and indoor components that include people, inanimate surfaces, substances, food, indoor air, waste and water systems [1, 2]. Although endemic transmission of HCAIs on surfaces has been suggested as playing an insignificant role [3], these sites are known to be contaminated by important nosocomial pathogens, notably *Pseudomonas* aeruginosa [4].

P. aeruginosa is a ubiquitous, versatile pathogen endowed with formidable virulence [5]. In addition, this rod is the leading cause of high mortality rates from hospital-acquired infections in critically affected patients [6], especially in the ICU [7]. Despite a wide distribution in nature, *P. aeruginosa* has been identified as the most frequently isolated non-fermenting gram-negative bacilli in the hospital environment, accounting for one third of all HCAIs in Brazil [8].

Additionally, *P. aeruginosa* has been one of ten leading pathogens to cause nosocomial pneumonia, urinary tract infections, surgical site infections, bloodstream infections and ventilator-associated pneumonia for the last four decades [9, 10]. These cases are more severe when *P. aeruginosa* specimens are multi-drug resistant [11].

The role of the hospital environment in harboring and transmitting multidrug-resistant organisms is linked to the increased risk of HCAIs [12]. Potential pathogens are able to transfer from the outside environment to the hospital, and the versatility of these pathogens favors the acquisition of characteristics that make them a serious public health concern [13]. The incidence of antimicrobial resistance is a emerging phenomenon worldwide [14]. Given this, a hospital environment can be said to contribute greatly to the spread of *P. aeruginosa* [15].

Hospital water is an acknowledged source of *P. aeruginosa* contamination [16]. with this bacteria as the leading cause of many outbreaks attributed to contamination of water systems [17-20]. This means that there must be effective and responsible care of the water as well as the effluents from hospitals, including material disinfected with chlorine [21], given that *P. aeruginosa* can survive in chlorinated [22], cold [23] and warm water [24] because it is able to colonize biofilms in piping systems [25]. The pipes ensure nutrient availability and promote persistence as a function of developing tolerance to various compounds present in subinhibitory concentrations in water [26].

Based on the relevance of the wide use of water in the hospital environment [27], as well as recognizing water as an important vehicle for the transmission of HCAIs [28, 29]. this work reports on the presence of *P. aeruginosa* and coliforms in water samples collected at a public hospital during a 16-week monitoring period.

MATERIAL AND METHODS

Description of the hospital's supply system and collection sites

The public hospital has two water sources: the first source is from its own wells, passing through a water treatment plant (WTP). The second source of water is from storage tanks (ST) where water obtained through purchase and transport by water trucks is maintained to exclusively serve the hemodialysis unit. The hospital water management system aims to guarantee the water demand for the entire hospital despite supply problems faced by the municipality, particularly during the dry season.

For 16 weeks, samples of 1000 mL of water were collected by aseptic means directly from the taps in five units: adult ICU (ICU-A), neonatal ICU (ICU-N), water treatment plant (WTP), storage tank (ST) and the hemodialysis unit. The collection protocol was performed according to the 9060 method of APHA et al [30].

Microbiological testing of water

The following parameters were determined: *P. aeruginosa* count (method 9213F), detection of total coliforms (method 9221D) and detection of thermotolerant coliforms (method 9221F) [30].

pH determination

The pH of the samples was determined by potentiometry using the ISO 10523:2008 method [31].

Isolation of pyocyanin-producing *Pseudomonas aeruginosa* (PYO⁺)

From each tube containing positive samples for *P. aeruginosa*, a 10 µL aliquot was spread over the surface of cetrimide agar spread in Petri dishes. After 18h of incubation at 35ºC, the presence of typical colonies and pigment diffusion in the agar was observed [32]. These isolates (PYO⁺) were subcultured on nutrient agar to perform the antibiogram.

Antibiogram

The PYO⁺ isolates were tested for their susceptibility to 17 antibiotics employed in the empirical antipseudomonal therapy scheme by using Kirby-Bauer disk diffusion method [33].

RESULTS

Table 1 summarizes the results of the microbiological analyses of the water samples. There is a large population of *P. aeruginosa* in the WTP samples, ranging between 93 and 460 MPN/100mL. In the ST, on the other hand, the bacterium was detected only in the first month of monitoring. In the hemodialysis unit, however, the samples were negative for *P. aeruginosa*.

In terms of coliform analyses, there was a greater presence of total coliforms than thermotolerant ones and once again the samples from the WTP were higher than potability standards. The samples presented greater contamination when compared to samples from the ST. It was also observed that coliform bacteria were absent when the most probable number of *P. aeruginosa* was very high and they were present when number of *P. aeruginosa* was zero. This pattern was not always observed, as the case of water samples from the WTP.

Table 1: Detection of pathogens in the effluent water

Weeks	Microbiological parameters/collection site			
	Pseudomonas aeruginosa (MPN/100mL)		Total coliform/Thermotolerant	
	WTP	ST	WTP	ST
1-4	240	75	– / –	– / –
5-8	460	0	+ / +	+ / +
3-12	93	0	+ / –	+ / –
13-16	240	0	+ / –	– / –

WTP – water treatment plant, ST – storage tank

There was a considerable variation in *P. aeruginosa* density in samples collected from ICU-A taps (between 28 and 240 MPN/100mL) and ICU-N (between absent and >2400 MPN/100mL). Coliform bacteria however were not detected in those samples, and water was considered potable in terms of bacteriological contamination. In addition, the pH averages throughout the monitoring were 7.9±0.1 (ICU-A), 7.0±0.2 (ICU-N), 6.2±0.3 (WTP) and 6.9±0.1 (ST).

A total of 12 PYO⁺ specimens were recovered. Most were isolated from the ICU-A (7) taps, followed by the WTP (3), ICU-N (1) and ST (1) taps. Submitted to antimicrobial susceptibility testing (Table 2), all specimens demonstrated multidrug-resistance, ranging from 4 (ICU-A) to 12 (ICU-N) of the 17 antibiotics tested. In addition, three specimens (25%) exhibited resistance to between 4 and 6 antibiotics and nine specimens (75%) demonstrated resistance to between 7 and 12 antibiotics, (mode = 7, for all recovered PYO⁺ from WTP and one PYO⁺ from the ICU-A tap). Most *P. aeruginosa* specimens were resistant to beta-lactam antibiotics and all were sensitive to quinolones and cefepime.

Table 2: Number and frequency of antimicrobial resistant pyocyanin-producing *Pseudomonas aeruginosa* recovered from water samples (n = 12)

Antibiotic	Specimens	
	n	**%**
CFZ	11	91.7
CFO	11	91.7
CLO	11	91.7
NIT	11	91.7
NAL	11	91.7
TET	9	75.0
SUT	6	50.0
MPM	4	33.3
IPM	4	33.3
CFX	4	33.3
AMI	1	8.3
GEN	1	8,3
TOB	1	8.3
ATM	1	8.3
NOR	0	0.0
CIP	0	0.0
CPM	0	0.0

DISCUSSION

Risk-management approaches to water safety in hospitals are designed to avoid potential hazards from waterborne HCAIs both in water supply and distribution [34]. HCAIs and outbreaks are usually correlated with water sources.[35] *P. aeruginosa* and a wide variety of other opportunistic pathogens can colonize biofilms on the surface of pipes, making them directly responsible for dissemination of HCAIs [36-38].

The ubiquitous nature of *P. aeruginosa* as well as its ability to grow in an oligotrophic environment allows the bacterium to occur in water from diverse sources [39, 40]. This is a major concern in hospitals [41] because it is associated with an increased risk of severe infections in the vulnerable population, particularly victims of burns and immunocompromised [42].

E. coli, on the other hand, is considered a truly fecal coliform [43]. as it has its exclusive habitat in the intestine of warm-blooded animals. Thus, its presence is a strong indication of recent fecal contamination, whether by sewage or animal waste contamination [44].

In contrast, although coliform bacteria are recognized as the most important index of water microbial quality, a large variation in the detection of coliforms can be detected in a single monitoring [45, 46], suggesting that this parameter alone is not sufficient to guarantee the consumption of safe water [47]. Variations in detections of microbiological parameters in water are expected when monitoring is often made with a limited number of samples [48]. It is important to consider that the variability related to sampling protocols is associated with two main types of factors: temporal variability (temperature, time of day or effluent discharge) and spatial variety (flow, depth and angle of collection) [49].

Limitations of this study are acknowledged. The water stored in the ST was from an external source, being used as an additional supply to the facility's needs. Since the transport of water to the hospital is carried out by water truck, multifactorial external conditions should be considered with respect to the variations in the results of microbial detection of the water samples, such as indicator density associated with rain [50] and differences in the residual chloride decay within the water tanker trucks [51].

In addition, traditional quantification methods are questioned as to their sensitivity to microbial cell interference or antagonistic substances [52]. False negatives may occur due to suppression of *E. coli* and other coliforms as the result of excessive growth of *P. aeruginosa* [53].

The main factor involved in the antagonistic relationships between *P. aeruginosa* and *E. coli* and other coliforms is the secretion of pyocyanin, a blue-colored phenazine, synthesized by about 90-95% of *P. aeruginosa* strains [54]. Phenazine-producing microbes are dominant and ecologically competent in the environment because there is a correlation between the ability to synthesize phenazine and persistence in hostile sites, such as the hospital environment [55]. A previous study found that 95% of all hospital isolates of *P. aeruginosa* produced pyocyanin and this finding was also correlated with the virulence of the specimens [56].

Pyocyanin is individually secreted at basal levels from a minimal number of cells. Additionally, pyocyanin production is modulated by a coordinated system of gene expression in response to fluctuations in cell-population density [57]. Pyocyanin easily interacts with the cell membrane. The mechanism of susceptible cell inhibition is oxidative stress through electron flow and intracellular accumulation of reactive oxygen species, mainly superoxide and hydrogen peroxide.[58] With this, pyocyanin inhibits growth in concentrations that vary widely from one species to another among susceptible organisms. Some studies, however, suggest a biostatic effect, even in low concentrations, against filamentous fungi [59], yeast [60], and bacteria [61].

On Enterobacteriaceae, pyocyanin may exhibit three response effects: the bactericidal or bacteriostatic effect on the planktonic population [62], as well as the disturbance of adhesion to surfaces, interfering with the subsequent colonization of biofilms [63], even in subinhibitory concentrations [64]. In a long-term stationary phase, however, both *P. aeruginosa* and *E. coli* synthesize exometabolites that mutually inhibit the exposed cells, ensuring the balance of both populations in aqueous media [65]. which may justify the presence of total and fecal coliforms in some samples. Under stress, *E. coli* can produce indole and acetate [66-68], but indole is more active against *P. aeruginosa*. In two recent studies, a reduction of more than 50% of pyocyanin production was observed when *P. aeruginosa* isolates were exposed to concentrations from up to 0.5 mM of indole [69]. In addition, this compound in concentrations of 0.5 and 1.0 mM disturbed the adhesion of *P. aeruginosa*, and reduced the stability of mature biofilms. However, *E. coli* appears to be more sensitive to pyocyanin than *P. aeruginosa* to indole [70].

Although pyocyanin production contributes to the increased expression of other virulence factors in *P. aeruginosa*, antibiotic multi-resistance does not appear to be associated with pyocyanin production [71]. This is because the mechanisms that lead to the resistance pattern in *P. aeruginosa* are multifactorial [72]. Still, PYO_+ strains exhibit a higher prevalence of multidrug-resistance and multiple virulence factors when compared to non-pyocyanin-producing strains [58], possibly because pyocyanin is a cellular signaling molecule [73].

On the other hand, *P. aeruginosa* is recognized for using its intrinsic and adaptive mechanisms to defeat most antibiotics [74]. These mechanisms include restriction of membrane permeability, efflux systems and production of antibiotic-inactivating enzymes [75]. In addition to the selective pressures to which *P. aeruginosa* strains are exposed in a hospital environment, the acquired resistance to multiple antibiotic classes should also be considered [76].

As far as possible, the present work was designed to be a controlled study. Despite temporal variability during monitoring, the results indicate a general risk of opportunistic infections from the use of water for different activities. Additionally, the study highlights the need for a stricter assessment of the effectiveness of water treatment and storage protocols, as well as periodic maintenance of water distribution systems. These are great challenges to hospital management, however, feasible [77].

CONCLUSION

From water samples collected at different points within a public hospital complex, several specimens of PYO^+ and resistant to multiple classes of antibiotics *P. aeruginosa* were isolated, highlighting the risk of HCAIs in patients admitted to the ICU. In addition, the detection of coliforms in samples collected from a few points around this hospital provides an alert to the need for routine implementation of a stricter control of the effectiveness of the treatment procedures and distribution of all hospital water.

The findings also suggest the importance of discussing the inclusion of additional parameters in the analysis of water quality for hospital use. The detection of *P. aeruginosa*, for example, should be considered as a complement to the regular detection of coliforms. Considering a water as potable based

only on the absence of coliforms does not guarantee the safety of the water, since opportunistic agents that are not included in the standards as indicators of health risk can use the water as a vehicle for contamination.

From these findings it is possible to deduce that many vulnerable patients in a hospital environment are exposed to biological risks with water acting as a good vehicle. It is essential to emphasize that the directives that control the bacteriological quality of drinking water are based on needs for people who are generally healthy. The hospital environment, however, hosts a vulnerable population that can evolve from disease or injury to death from an HCAI related to direct or indirect contact with water contaminated by opportunistic agents. Therefore, it is not excessive to suggest the elaboration of a specific directive that regulates water quality standards for hospital supply.

AKNOWLEDGMENTS

The authors would like to thank the hospital for its support by allowing this work to be carried out. The English text of this paper has been revised by Sidney Pratt, Canadian, MAT (The Johns Hopkins University), RSAdip - TESL (Cambridge University).

REFERENCES

[1] Bonadonna L, Briancesco R, Coccia AM. Air Quality Healthcare Fac 2017; 53-62.
[2] Oumokhtar B, Lalami AEO, Benaicha N, Arhoune B, Bono W. Biomedical Res 2017; 28: 2398-2401.
[3] Otter JA, Yezli S, French GL. Infect Control Hosp Epidemiol 2011; 32: 687-699.
[4] Güdücüoğlu H. Eastern J Med 2015; 20: 177-181.
[5] Kerr KG, Snelling AM. J Hosp Infect 2009; 73: 338-344.
[6] Matos ECO, Andriolo RB, Rodrigues YC, Lima PDL, Carneiro ICRS, Lima KVB. Rev Soc Bras Med Trop 2018; 51: 415-420.
[7] Harris AD, Jackson SS, Robinson G, Pineles L, Leekha S, Thom KA, Wang Y, Doll M, Pettigrew MM, Johnson JK. Infect Control Hosp Epidemiol 2016; 37: 544-548.
[8] Braga IA, Campos PA, Gontijo-Filho PP, Ribas RM. J Hosp Infect 2018; 99: 318-324.
[9] Lister PD, Wolter DJ, Hanson NC. Clin Microbiol Rev 2009; 22: 582-610.
[10] Trautmann M, Lepper PM, Haller M. Am J Infect Control 2005; 33: S41-S49.
[11] Brzozowski M, Krukowska Ż, Galant K, Jursa-Kulesza J, Kosik-Bogacka D. BMC Infect Dis 2020; 20: 693, doi: 10.1186/s12879-020-05404-w.
[12] Chemaly RF, Sarah S, Charles D, Shashank S, Ghantoji S, Maria R, Julie G, Julie S, Mark S. J Ther Adv Infect Dis 2014; 2: 79-90.
[13] Suleyman G, Alangaden G, Bardossy AC. Curr Infect Dis Rep 2018; 20: 12, doi: 10.1007/s11908-018-0620-2.
[14] Chokshi A, Sifri Z, Cennimo D, Horng H. J Glob Infect Dis 2019; 11: 36-42.
[15] Oliveira AC, Damasceno QS. Rev Esc Enf USP 2010; 44: 1112-1117.
[16] Walker J, Moore G. J Hosp Infect 2015; 89: 324-327.
[17] Garvey MI, Bradley CW, Holden E. Am J Infect Control 2018; 46: 383-386.
[18] Walker J, Moore G. J Hosp Infect 2016; 94: doi 10.1016/j.jhin.2016.07.001.
[19] Costa D, Bousseau A, Thevenot S, Dufour X, Laland C, Burucoa C, Castel O. J Hosp Infect 2015; 91: 271-274.
[20] Muscarella LF. Infect Control Hosp Epidemiol 2004; 25: 342-345.
[21] Azuma T, Hayashi T. Sci Total Environ 2021; 776: 145951, doi: 10.1016/j.scitotenv.2021.145951.
[22] Marshal C, Denton S, Christie I, Orr L. Infect Control Hosp Epidemiol 2020; 41(S1): doi: 10.1017/ice.2020.745.
[23] Peter A, Routledge E. Plos One 2018; 13: doi 10.1371/journal.pone.0195635.
[24] Moritz MM, Flemming H-C, Wingender J. Int J Hyg Environ Health 2010; 213: 190-197.
[25] Suman E, Singh S, Kotian S. J Hosp Infect 2008; 70: 199-201.
[26] Voumard M, Venturelli L, Borgatta M, Croxatto A, Kasas S, Dietler G, Breider F, von Gunten U. Environ Sci Water Res Technol 2020; 6: 1139-1151.
[27] Topbas M, Beyhun NE, Can G, Ucuncu SY, Kolayli CC, Karakullukcu S, Cankaya S. J Environ Protect Ecol 2016; 17: 772-780.
[28] Decker BK, Palmore TN. Curr Opin Infect Dis 2013; 26: 345-351.
[29] Kusnetsov J, Torvinen E, Perola O, Nousiainen T, Katila M-L. APMIS; 2003; 111: 546-556.
[30] APHA, AWWA, WEF. Standard Methods for the Examination of Water and Wastewater 2012.

[31] ISO 10523 Water quality – Determination of pH 2008.
[32] Lowburry EJL, Collins AG. J Clin Pathol 1955; 8: 47-48.
[33] NCCLS. Methods for dilution Antimicrobial Susceptibility Tests for bacteria that grow aerobically 2003.
[34] Danila RC, Koranteng N, Como-Sabetti KJ, Robinson TJ, Laine ES. Infect Control Hosp Epidemiol 2018; 39: 336-338.
[35] Moghadam MAJ, Honarmand H, Meshginshahr SA. Interdiscip Perspect Infect Dis 2015; 2015: e809842, doi: 10.1155/2015/809842.
[36] Shamsizadeh Z, Ehrampoush MH, Nikaeen M, Ebrahimi AA, Asghari FB. Int J Env Health Eng 2020; 9:10, doi: 10.4103/ijehe.ijehe_12_19.
[37] Bédard E, Prévost M, Déziel E. Microbiol Open 2016; 5: 937-956.
[38] Loveday HP, Wilson JA, Kerr K, Pitchers R, Walker JD, Browne J. J Hosp Infect 2014; 86: 7-15.
[39] Briancesco R, Paduano S, Semproni M, Vitanza L, Bonadonna L. Environ Res Public Health 2020; 17: 8263, doi: 10.3390/ijerph17218263.
[40] Caskey S, Stirling J, Moore JE, Rendall JC. Lett Appl Microbiol 2018; 66: 537-541.
[41] Pachori P, Gothalwal R, Ghandi P. Gene Dis 2019; 6: 109-119.
[42] Karami P, Mohajeri P, Mashouf RY, Karami M, Yaghoobi MH, Dastan D, Alikhani MY. Saudi J Biol Sci 2019; 26: 1731-1736.
[43] Cabral JP, Marques C. Environ Monit Assess 2006; 118: 21-36.
[44] Alam JB, Hossain A, Khan SK, Banik BK, Islam MR, Muyen Z, Rahman MH. Environ Monit Assess 2007; 134: 233–242.
[45] Singh AK, Das S, Singh S, Padhamn N, Gajamer VR, Kumar S, Lepcha YD, Tiwari HK. Front Public Health 2019; 7: 174, doi: 10.3389/fpubh.2019.00174.
[46] Bumadian MM, Almansury HH, Bozakouk IH, Lawgali YF, Bleiblo FA. J Exp Biol Agr Sci 2013; 1: 436-440.
[47] Deshmukh RA, Joshi K, Bhand S, Roy U. Microbiol Open 2016; 5: 901–922.
[48] Kozuskanich J, Novakowski KS, Anderson BC. Ground Wat 2011; 49: 43-52.
[49] Åström J, Pettersson TJ, Stenström TA, Bergstedt O. Water Sci Technol 2009; 59: 203-212.
[50] Silva CR, Sanches MS, Milhim BHGA, Rocha SJD, Pelayo JS. Semina Ciênc Biol Saúde 2019; 40: 129-140.
[51] Ecuru J, Okot-Okumu J, Okurut TO. JASEM 2011; 15: 167-173.
[52] Carrillo-Gómez J, Durán-Acevedo C, García-Rico R. Water 2019; 11: 774, doi: 10.3390/w11040774.
[53] Vasconcelos U, Lima MAGA, Calazans GMT. Can J Pure Appl Sci 2010; 4: 1133-1139.
[54] Mavrodi D, Bonsall RF, Delaney SM, Soule MJ, Phillips G, Thomashow LS. J Bacteriol 2001; 183: 6454–6465.
[55] Mazzola M, Cook RJ, Thomashow LS, Weler DM, Person III LS. Appl Environ Microbiol 1992; 58: 2616–2624.
[56] Nowroozi J, Sepahi AA, Rashnonejad A. Cell J 2012; 14: 7–18.
[57] Al-Shabib NA, Husain FM, Khan RA, Khan MS, Alam MZ, Ansari FA, Laeeq S, Zubair M, Shahzad SA, Khan JM, Alsami A, Ahmad I. Saudi J Biol Sci 2019; 26: 308–316.
[58] Gonçalves T, Vasconcelos U. Molecules 2021; 26: 927, doi: 10.3390/molecules26040927.
[59] Silva JEG, Bonifácio TTC, Arruda RRA, Kretzschmar EAM, Vasconcelos U. Int J Develop Res 2020; 10: 35230-35234.
[60] Bonifácio TTC, Arruda RRA, Oliveira BTM, Silva JEG, Vasconcelos U. Res J Pharm Biol Chem Sci 2020; 11: 111-119.
[61] Guilherme EFM, Silva JAM. Rev Hig Alim 2000; 14: 43-47.
[62] Martins VV, Macareno AC, Gradella DG, Stehling EG. Water Sci Technol Water Supply 2014; 14: 99-106.
[63] Tait K, Sutherland IW. J Appl Microbiol 2002; 93: 345-352.
[64] Viana AAG, Martins RX, Ferreira GF, Zenaide-Neto H, Amaral IPG, Vasconcelos U. Int J Eng Res Appl 2017; 7: 23-30.
[65] Kuznetsova MV, Maslennikova IL, Karpunina TI, Nesterova LY, Demakov VA. Can J Microbiol 2013; 59: 604–610.
[66] Gaimster H, Cama J, Hernández-Ainsa S, Keyser UF, Summers DK. PLoS One 2014; 9: e93168, doi: 10.1371.
[67] Chu W, Zere TR, Weber MM, Wood TK, Whiteley M, Hidalgo-Romano B, Valenzuela EJ, McLean RJC. Appl Environ Microbiol 2011; 78: 411–419.
[68] Kleman GL, Strohl WR. Appl Environ Microbiol 1994; 60: 3952-3958.

[69] Arruda RRA, Oliveira BTM, Bonifácio TTC, Morais VC, Amaral IPG, Vasconcelos, U. Int J Adv Eng Res Sci 2019; 6: 267–271.
[70] Arruda RRA, Bonifácio TTC, Oliveira BTM, Silva JEG, Vasconcelos U. Int J Develop Res 2020; 10: 34122-34128.
[71] Finalayson EA, Brown PD. West Indian Med J 2011; 60: 24–32.
[72] Meliani A. Exp Clin Sci J 2020; 19: 813-816.
[73] Dietrich LEP, Price-Whelan A, Petersen A, Whiteley M, Nwman DK. Mol Microbiol 2006; 61: 1308–1321.
[74] Martins RX, Viana AAG, Ferreira GF, Cavalcanti TG, Amaral IPG, Travassos RA, Vasconcelos U. J Appl Pharm Sci 2018; 8: 169-174.
[75] Pang Z, Raudonis R, Glick BR, Lin T-J, Cheng Z. Biotechnol Adv 2019; 37: 177-192.
[76] Paramythiotou E, Lucet J-C, Timsit J-F, Vanjak D, Paugam-Burtz C, Trouillet J-L, Belloc S, Kassis N, Karabinis A, Andremont A. Clin Infect Dis 2004; 38: 670–677.
[77] Chirca I. Fut Microbiol 2019; 14: doi: 10.2217/fmb-2019-0140.