Aptamers and Aptamer-Coupled Biosensors to Detect Water-Borne Pathogens

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Aptamers can serve as efficient bioreceptors for the development of biosensing detection platforms. Aptamers are short DNA or RNA oligonucleotides that fold into specific structures, which enable them to selectively bind to target analytes. The method used to identify aptamers is Systematic Evolution of Ligands through Exponential Enrichment (SELEX). Target properties can have an impact on aptamer efficiencies. Therefore, characteristics of water-borne microbial targets must be carefully considered during SELEX for optimal aptamer development. Several aptamers have been described for key water-borne pathogens. Here, we provide an exhaustive overview of these aptamers and discuss important microbial aspects to consider when developing such aptamers.

Keywords: aptamer, SELEX, water-borne pathogens, viable but non-culturable, coliforms, aptasensors

INTRODUCTION

Access to water that is safe for use and consumption is a basic human right. As a result, most countries have strict guidelines, regulations and standards for managing water sources and water distribution systems to supply high quality water free from chemical and microbial contaminants. In most cases, microbial contaminants must be removed from the water before distribution. These microbes include pathogens that cause gastroenteritis, such as Cryptosporidium, Giardia, Norovirus, Rotavirus, Campylobacter, and E. coli (WHO, 2017). Other water-borne diseases are caused by pathogens growing inside water distribution systems or within engineered water systems, such as cooling tower, fountains, spas and humidifiers (Wang H. et al., 2017). The latter include Legionella pneumophila, Pseudomonas spp. and non-tuberculosis mycobacteria. In recent years, several studies have shown that a high proportion of water associated deaths and illnesses are due to the aforementioned three environmental water-borne pathogens (Gargano et al., 2017; Greco et al., 2020). In fact, L. pneumophila, the causative agent of Legionnaires disease, has become the number one cause of water-borne outbreaks in recent years (McClung et al., 2017). The presence of coliforms is not indicative of the presence of several key water-based pathogens that are of significance to public health (Payment and Locas, 2011). Consequently, specific detection methods are needed to ensure safe water from the source to the point of use.
Monitoring and surveillance of specific water-borne microbes require robust detection methods. Challenges in select current detection methods for waterborne pathogens have been reviewed excellently in detail elsewhere (Ramírez-Castillo et al., 2015; Wang et al., 2017). In general, traditional microbial detection methods rely heavily on culture methods, which is fraught with several limitations. Culture methods are extremely time consuming and often require extensive material, specialized labor, and time. Culture recovery rates are also adversely affected by many factors such as the presence of competing microbes, the presence of viable but non-culturable (VBNC) cells, methods used for concentration of the sample or enrichment of the target microbe and sample type (bulk water or biofilm) (Wang et al., 2017). Drawbacks with culture techniques has led to a shift toward the use of molecular methods, including PCR, quantitative PCR (qPCR), high throughput sequencing, and immunoassays such as ELISA, immunochromatography and immuno-lateral flow assays. The most widely used molecular method is qPCR (Ramírez-Castillo et al., 2015; Wang et al., 2017). The advantage of qPCR, over conventional culture techniques, is more rapid turn-around times, high sensitivities and specificities, lower limits of detection, as well as an ability to detect VBNC cells. However, by detecting live, VBNC and dead cells qPCR leads to an overestimation of microbial burden. Additionally, qPCR involves multiple sample processing steps which requires specialized labor. qPCR is also inhibited by several compounds routinely found in water samples resulting in possible false negatives (Gentry-Shields et al., 2013).

Biosensors can mitigate some of the problems associated with traditional detection methods (Ahmed et al., 2014). They are analytical devices used to quantify or detect a specific analyte (Turner, 2013). Qualities of biosensors includes high specificity, high sensitivity, multiplexing capability, cost-effectiveness, portability and ease of use (Ahmed et al., 2014; Kumar et al., 2018; Cesewski and Johnson, 2020; McConnell et al., 2020). A biosensor set-up typically consists of three elements. A biorecognition element, which upon interaction with a target, produces a physico-chemical signal that is converted by a transducing element into a signal captured by a detection element (Turner, 2013). Biosensors are categorized based on either their transducing element (mechanical, optical, electrochemical) or the nature of the biorecognition element (affinity, catalytic) (Ahmed et al., 2014).

A versatile and stable biorecognition element is a critical component of any biosensing platform (Ahmed et al., 2014; Kumar et al., 2018). Antibodies are the most used bioreceptors in biosensor development and research, but aptamers are an increasingly widespread popular alternative (Song et al., 2008; Morales and Halpern, 2018). Aptamers are single stranded DNA or RNA oligonucleotides that fold into specific complex structures and interact with their targets via shape complementarity, hydrogen bonding, electrostatic interactions and stacking interactions (McKeague et al., 2015). Besides having high affinities and selectivity, they can bind to a wide range of targets from small non-immunogenic compounds to whole cells (McKeague et al., 2015). Aptamers can be generated in vitro in conditions one can preferentially select making them stable and versatile for a variety of applications (Song et al., 2008). They are cost-effective to synthesize with minimal batch to batch variation (Strehlitz et al., 2012; McConnell et al., 2020). Their easily modifiable nature facilitates functionalization on sensing surfaces (Song et al., 2008; McConnell et al., 2020). Their inherent small size also promotes high packing densities during functionalization (Song et al., 2008; Crivianu-Gaita and Thompson, 2016). In this minireview, we will briefly provide examples of aptamers with potential for detection of water-borne pathogens and discuss microbial determinants for the development of optimal aptamers and thus improved aptamer-coupled biosensors. Examples of aptamers is provided in Table 1 and a complete list of aptasensing platforms is provided in Supplementary Table 1.

**APTAMER DEVELOPMENT**

Aptamers are typically identified by SELEX (Systematic Evolution of Ligands through Exponential Enrichment). SELEX is an iterative process where repeated exposure of a target to a large pool of random oligonucleotides results in the gradual enrichment of specific sequences that bind with the highest affinity to the target. Since the technique's inception in 1990, many variations of the original SELEX method have been published (Darmostuk et al., 2015). These experimental variations differ based on desired aptamer properties and details have been reviewed elsewhere (Wang et al., 2019). Of note, cell-SELEX can be used to select aptamers against whole cells in solution, to ensure cell surface target epitopes are in their native state (Kaur, 2018). This method is particularly useful for developing aptamers to detect water-borne pathogens. Cell-SELEX may include counter-selection steps to remove sequences binding to non-target microbes thus minimizing cross-reactivity and improving the specificity of the resulting aptamers (see Table 1 for examples).

Several aptamer-coupled biosensing systems or aptasensors have been described for the detection of water-borne pathogens or toxins accumulating in water (Table 1 and Supplementary Table S1) with the majority targeting bacterial pathogens. Nevertheless, none have been officially adopted for routine detection of water-borne pathogens. The development of successful aptamer-coupled biosensors to detect water-borne pathogens requires a multi-pronged approach. Besides intricate knowledge of the sensing system, its transducer, the physico-chemical phenomenon that mediate signal responses, and a deep understanding of aptamer chemistries, careful consideration of the physiology and ecology of the target microorganism is required. This is because physio-ecological factors affect microbial morphologies and surface structures and thus the presence of aptamer targets (Figure 1). Although several works discuss transducing systems and aptamer design and chemistries in detail, relatively fewer studies consider the physio-ecological context of water-borne microbes for sensing platforms. Since most aptamers and aptasensing systems described in the literature detects water-borne bacterial pathogens, properties of bacteria are discussed in more detail to illustrate the importance.
# TABLE 1 | Aptamers developed against water-borne bacteria.

| Aptamer name | Target | Culture condition | OD/Growth stage | Counter-Selex Strains | Type of sensors | LOD | References |
|--------------|--------|-------------------|-----------------|-----------------------|-----------------|-----|------------|
| Norovirus    |        |                   |                 |                       |                 |     |            |
| AG3          | MuNoV  | NA                | NA              | Feline calicivirus (FCV) | Electrochemical | 180 virus particles | Giamberardino et al., 2013 |
| Aptermer     | SMV    | NA                | NA              | HuNoV-negative human stool suspension, bead-antibody complex | Optical (colorimetric) | NA | Weerathunge et al., 2019 |
| 25/SMV-25    |        |                   |                 |                       |                 |     |            |
| Aptermer     | SMV    | NA                | NA              | HuNoV-negative human stool suspension, bead-antibody complex | Optical (Chemiluminescence) | NA | Escudero-Abarca et al., 2014 |
| 21/SMV-21    |        |                   |                 |                       |                 |     |            |
| Norovirus Group II (recombinant VLP) |        |                   |                 |                       |                 |     |            |
| C. parvum    | Oocysts | NA                | NA              | Giardia duodenalis cysts | Electrochemical | 100 oocysts | Iqbal et al., 2015 |
| Min_Crypto2  | Oocysts | NA                | NA              | Giardia duodenalis cysts | Electrochemical | 50 oocysts | Iqbal et al., 2019 |
| Acinetobacter| Ac49    | Whole-cell-A. baumannii (ATCC 19606) | BHI broth, 37°C, overnight | 0.4/E | Acinetobacter lwoffii, Acinetobacter calcoaceticus, and 11 species | Optical (colorimetric) | 10² CFU/ml | Rasoulinejad and Gargari, 2016 |
|               |        |                   |                 |                       |                 |     |            |
| C. jejuni     | Oocysts | NA                | NA              |                       | Optical (fluorescence) | 100 CFU/ml | Su et al., 2020 |
| ONS-23       | Whole-cell (C. jejuni/ A9a) | BBL brucella broth, 42°C, 48 h, microaerophilic conditions | PE* 20 strains (enteric, non-enteric, lactic acid) |                   | Optical (fluorescence) | 10 CFU/ml | Dehghani et al., 2018 |
| CJA1         | Whole-cell (C. jejuni) |                   |                 |                       | Optical (colorimetric) | 7.2 x 10⁷ CFU/ml | Kim Y. J. et al., 2018 |
|              |        |                   |                 |                       | Optical (colorimetric) | 10 CFU/ml | Chen et al., 2020 |

(Continued)
TABLE 1 | Continued

| Aptamer name | Target | Culture condition | OD/Growth stage | Counter-Selex Strains | Type of sensors | LOD | References |
|--------------|--------|-------------------|-----------------|-----------------------|----------------|-----|------------|
| Cyanobacteria |        |                   |                 |                       |                |     |            |
| ATX8         | Anatoxin-a (ATX) | NA | NA | ATX free beads | Electrochemical | 0.5 nM | Elshafey et al., 2015 |
| MC-LR aptamer/AN6 | Microcystin-LR | NA | NA | Blank sepharose beads | Electrochemical | 10 pM | Ng et al., 2012 |
| E. coli     | L9F    | O111-LPS (E. coli O111:K58) | 35°C, TSB, overnight | NR | Electrochemical | 0.002 ng/ml | Lv et al., 2017 |
| Eco4R/ECAI  | Outer membrane protein (OMP)—E. coli 8739 | 37°C, blood agar, overnight | NR | NA | Optical (fluorescence) | 112 CFU/ml | Luo et al., 2012 |
| Eco4F       | OMP-E. coli 8739 | 37°C, blood agar, overnight | NR | NA | Electrochemical | 300 CFU/ml | Bruno et al., 2008 |
| Eco3R/ECAI  | OMP-E. coli 8739 | 37°C, blood agar, overnight | NR | NA | Optical (colorimetric/fluorescence) | 300 CFU/ml | Queirós et al., 2013 |
| E1          | Whole cell (E. coli fecal isolate) | NB, 37°C | 0.45/E | E. coli (non-fecal isolate), other fecal isolates | Optical (fluorescence) | 1.1 x 10³ CFU/ml | Peng et al., 2014 |
| E2          | Whole cell (E. coli fecal isolate) | NB, 37°C | 0.45/E | E. coli (non-fecal isolate), other fecal isolates | Optical (fluorescence) | 10 CFU/ml | Lee et al., 2009 |
| E10         | Whole cell (E. coli fecal isolate) | NB, 37°C | 0.45/E | E. coli (non-fecal isolate), other fecal isolates | Optical (fluorescence) | 6–26 CFU/ml | Zelada-Guilleìn et al., 2010 |
| E1 + E2 + E10 (pooled) |        |                   |                 |                       |                |     |            |
| AptB12      | Whole cell (E. coli ETEC K88) | LB | E | ETEC K99, S. enteritidis, S. aureus, | Optical (fluorescence) | 1.1 x 10³ CFU/ml | Peng et al., 2014 |
| RNAaptamer  | NR | LB, 37°C, 2–3 h | NR | NA | Electrochemical | 6–26 CFU/ml | Zelada-Guilleìn et al., 2010 |

(Continued)
| Aptamer name         | Target                          | Culture conditiona | OD/Growth stageb | Counter-Selex Strainsc | Type of sensors                  | LOD               | References               |
|---------------------|---------------------------------|--------------------|------------------|------------------------|----------------------------------|-------------------|--------------------------|
| Aptamer I-1         | O-antigen LPS (E. coli O157:H7) | Brucella broth, 37°C, 24 h (+0.04% formaldehyde) | NR               | E. coli K12            | NA                               | NA                | Lee et al., 2012         |
| Ec3 (31)            | Whole cell (E. coli DH5a)       | LB                 | 0.4              | NA                     | Electrochemical                  | 4 CFU/ml          | Burrs et al., 2016       |
| P12-31              | Whole cell (E. coli O6)         | 37°C, LB           | 0.3              | NA                     | Electrochemical                  | 2 x 10^6 CFU/ml   | Dua et al., 2016         |
| AM-6                | Whole cell (E. coli O157:H7)    | LB                 | 0.6              | E. coli strains O42, K12, Top10, DH5a, S. flexneri, S. Typh    | NA                 | NA                       | Marion et al., 2016     |
| S1                  | Whole cell (E. coli O157:H7)    | BHI, 37°C          | E                | NA                     | Mechanical (Quartz Crystal Microbalance-QCM) | 1.46 x 10^5 CFU/ml | Amraee et al., 2017      |
| Apt-5               | whole cell (E. coli O157:H7)    | LB, 37°C           | NR               | E. coli ETEC and 3 other species | NA                 | NA                       | Bruno et al., 2009       |
| a-aptamer/E-17F72*  | O157:H7 LPS                     | LB, 37°C           | NR               | NA                     | Optical (colorimetric)           | 10 CFU/ml         | Díaz-Amaya et al., 2019b |
| c-aptamer/E-18R72*  | O157:H7 LPS                     | LB, 37°C           | NR               | NA                     | Optical (colorimetric)           | 25 CFU/ml         | Díaz-Amaya et al., 2019a |
| a-aptamer, c-aptamer|                                 |                    |                  | NA                     | Optical (surface enhanced raman spectroscopy-SERS) | 100 CFU/ml        | Hao et al., 2019         |
| a-aptamer, c-aptamer|                                 |                    |                  | NA                     | Optical (fluorescence)           | 80 CFU/ml         | Jiang et al., 2020       |
| c-aptamer, c-aptamer|                                 |                    |                  | NA                     | Optical (fluorescence)           |                    |                          |
| c-aptamer           |                                 |                    |                  | NA                     | Optical (fluorescence)           |                    |                          |
| **Helicobacter pylori** |                                |                    |                  |                        |                                  |                   |                          |
| Hp-Ag aptamer       | Recombinant Hp surface antigen  | NR                 | NR               | BSA                    | NA                               | NA                | Gu et al., 2018          |
| Hp4                 | Recombinant Hp surface antigen  | Blood agar, 37°C, 3 days | NR               | BSA                    | NA                               | NA                | Yan et al., 2019         |
| **Legionella**      |                                  |                    |                  |                        |                                  |                   | Saad et al., 2020        |
| R10C5, R10C1        | Whole cell (Lp 120292)          | CYE agar plate, 37°C, 3 days followed by AYE media, 37°C, 24 h | 2.5/PE  | *Pseudomonas putida* KT2440, *Pseudomonas fluorescens* LMG1794 | NA                 | NA                       | Sun et al., 2016         |
| **NTM**             |                                  |                    |                  |                        |                                  |                   | Sodia et al., 2020       |
| BM2/N31             | ManLAM, M. bovis (BCG)          | L-J medium         | E                | NR                     | Optical (ELONA)                  | 10^6 CFU/ml       | Wang et al., 2011        |
| **Pseudomonas aeruginosa** |                                |                    |                  |                        |                                  |                   | Gao et al., 2018         |
| F23                 | Whole cell (P. aeruginosa clinical isolate) | Mueller-Hinton (MH) media, 37°C, 24 h | NR               | S. maltophilia, A. baumannii | Optical (fluorescence)           | NR                |                          |

(Continued)
| Aptamer name | Target | Culture condition | OD/Growth stage | Counter-Selex Strains | Type of sensors | LOD | References |
|-------------|--------|-------------------|-----------------|----------------------|-----------------|-----|------------|
| St17Lp21,  |
| St21Lp17,  |
| St08Lp17   |
| Biofilm-derived whole cells (PA 692/ATCC 14502) | LB broth, 37°C, 16 h followed by 22°C, 42 h to make biofilm. | E | NA | Optical (Long range Surface Plasmon Resonance-LSPR) | 1 CFU/ml | Hu et al., 2018 |
| F23 + St08Lp17 (pool) | NA | NA | NA | NA | 1 CFU/ml | Zhong et al., 2020 |
| Salmonella | OMP (S. typhimurium PT110) | BHI, 37°C, 2–3 h | E | NA | Optical (Fluorescence) | 1 CFU/ml | Zhong et al., 2020 |
| ST2P | Whole cell (S. typhimurium ATCC 50761) | BBL-BHI, 37°C, overnight | 0.3/E | E. coli OMP and LPS, Salmonella LPS | Magnetic bead based pull down assay and qPCR | 10–100 CFU/ml | Joshi et al., 2009 |
| S8-7 | Whole cell (S. typhimurium S913) | TSB-amp, 37°C, overnight | NR | L. monocytogenes Scott A E. coli O157: H7, B. cereus, E. faecalis E. coli, S. enteritidis, S. aureus | Optical (Fluorescence) | 10 CFU/ml | Duan et al., 2016 |
| C4 | Whole cell (S. typhimurium) | BHI, 35°C, overnight | NR | NA | Optical (Fluorescence) | 25 CFU/ml | Duan et al., 2014 |
| Apt22 | Whole cell (S. paratyphi A) | NB, 37°C | 2.1/E | S. Enteritidis, S. Typhimurium, S. Cholerasuis, S. Arizonae Salmonella serovars-multiple | Optical (chemiluminescence) | 1000 CFU/ml | Yang et al., 2013 |
| S25 | Whole cell (S. enteritidis-multiple) | TSB, overnight | NR | L. monocytogenes | Optical | 1000 CFU/ml | Hyeon et al., 2012 |
| SAL26 | Whole cell (S. typhimurium ATCC14028) | TSB, 37°C, overnight culture followed by TSB, 3 h then fixing with methanol | E | 4 Salmonella enterica serovars and 9 bacterial species. | Optical (Colorimetric) | 100 CFU/ml | Lavu et al., 2016 |

(Continued)
| Aptamer name | Target | Culture condition | OD/Growth stage<sup>a,b</sup> | Counter-Selex Strains<sup>c</sup> | Type of sensors | LOD | References |
|-------------|--------|------------------|-----------------|-----------------------------|----------------|------|------------|
| SAL1        | Whole cell (S. paratyphi-A ATCC 9150) | LB broth, 37°C | E               | S. Typhimurium, S. flexneri, E. coli O157:H7, Yersinia enterocolitica | Optical (fluorescence) | 10 CFU/ml | Rm et al., 2020 |
| B5          | Whole cell (S. typhimurium ATCC14028) | BHI broth, 37°C | PE              | S. aureus, L. monocytogenes, E. coli O157:H7 | Mechanical (QCM) | 1,000 CFU/ml | Wang L. et al., 2017 |
| Shigella    | Aptamer S 1 | Whole cell (Shigella dysenteriae) | LB | E | S. aureus, S. typhimurium, E. coli, L. monocytogenes, V. parahaemolyticus | Optical (Fluorescence) | 50 CFU/ml | Duan et al., 2013a |
|             | Sp1     | Whole cell (Shigella sonnei ATCC 51334) | LB, 37°C, overnight | NR | S. dysenteriae, S. flexneri, S. boydii, S. typhimurium, E. coli | NA | 1 CFU/ml | Zarei et al., 2018 |
|             | Sp20    | Whole cell (Shigella sonnei ATCC 51334) | LB, 37°C, overnight | NR | S. dysenteriae, S. flexneri, S. boydii, S. typhimurium and E. coli | NA | 10 CFU/ml | Wu et al., 2020 |
|             | S. flexneri aptamer1 | Whole cell (Shigella flexneri ATCC 12022) | LB, 37°C, overnight | NR | NA | Optical (fluorescence) | 100 CFU/ml | Zhu et al., 2015 |
|             | SS-3, SS-4 | Whole cell (Shigella sonnei) | NB, 37°C | NR | E. coli | Optical (Fluorescence) | 1,000 CFU/ml | Song et al., 2017 |
|             | S. flexneri aptamer2 | Whole cell (Shigella flexneri ATCC 12022) | NB, 37°C, 12 h | NR | NA | Optical (colorimetric) | 80 CFU/ml | Feng et al., 2019 |
| Vibrio cholerae | CT916 | Cholerae toxin | NA | NA | Ethanolamine-blocked magnetic beads | NA | Optical (colorimetric) | 2.1 ng/ml | Frohnmeyer et al., 2018 |
|             | Whole cell (V. cholerae O1-Inaba, ATCC 39315 and Ogawa) | LB broth, 37°C | 0.4/E | E. coli O157:H7, S. a dysenteriae, S. enteritidis, S. Typhimurium, Yersinia spp., S. flexneri | Optical (colorimetric) | 10<sup>6</sup> CFU/ml | Mojarad and Gargaria, 2020 |
| Yersinia    | N30yc5, N71yc2 | Recombinant Yop51 | NA | NA | NA | NA | NA | Bell et al., 1998 |
|             | M1, M5, M7 | Whole cell (Yersinia entercolitica) | Specific media (NaCl, beef extract, peptone, pH 7.2-7.4, 26°C | 0.3 (L), 0.6 (E), 0.9 (PE) | NA | NA | NA | Shoaib et al., 2020 |

<sup>a</sup> Microbial culture conditions and growth conditions are listed for aptamer development only. The Microbial culture and growth conditions used for aptasensors development are listed in Supplementary Table S1.

<sup>b</sup> State: L, lag phase; E, exponential; PE, post-exponential.

<sup>c</sup> If number of strains used for counter selection is higher than to 5, they are listed in Supplementary Table S1.
of considering the target's microbial characteristics for aptamer and aptasensor development.

**APTAMERS TARGETING MICROBES IN SPECIFIC STATES AND GROWTH CONDITIONS**

Protozoan microbes have varying life cycles which can alternate between metabolically active feeding states, i.e., trophozoites, or inactive, dormant states such as oocysts or cysts (Aguilar-Díaz et al., 2011; Jain et al., 2019). Both oocysts and cysts are infectious forms that persist for long periods of time in environmental waters and resist a wide range of stressors (Omarova et al., 2018). The Cryptosporidium parvum oocyst-specific aptamer R4-6 was thus developed using cell-SELEX (Table 1; Iqbal et al., 2015). A counter selection step against Giardia duodenalis, another protozoan commonly found in water samples (Ongerth, 2013; WHO, 2017) was included to enhance aptamer specificity. This aptamer was first used in multiple assay formats on electrochemical biosensing platforms to detect oocysts of C. parvum down to 50 oocysts in river and lake water samples (Iqbal et al., 2015; Iqbal et al., 2019). Recently, a fluorescence plate assay coupled with magnetic beads labeled with a truncated version of the aptamer R4-6, named Min Crypto2 achieved a detection limit of 5 oocysts (Hassan et al., 2021). The low LOD of this system is promising for oocyst detection in water given that the infectious dose of C. parvum is between 10 and 30 oocysts (Jain et al., 2019). Aptamer Min Crypto2 was selective for Cryptosporidium species, despite differences in size amongst species, but did not bind to Giardia oocysts. These features combined with its robust performance in water samples highlights its potential for oocyst detection in water.

Bacteria suspended in water are in a different metabolic state than bacteria growing in laboratory media. For example, L. pneumophila adopts a specific regulatory program when suspended in water due to starvation (Li et al., 2015). Consequently, the aptamers R10C5 and R10C1 were created by cell-SELEX using L. pneumophila suspended in water for 24 h, to allow the bacterium to adopt the associated metabolic state (Table 1). Counter selection was performed on two Pseudomonas spp. strains, prevalent in environmental waters (Paranjape et al., 2020). Both aptamers have excellent specificity for L. pneumophila (Saad et al., 2020).

Water borne bacteria can also be biofilm-associated. These bacteria can gain adaptive traits which make it harder to eliminate or disinfect them. To that end, biofilm-derived Pseudomonas aeruginosa cells were used to select aptamers through Cell-SELEX, without counter selection (Soundy and Day, 2017). The resulting aptamers were specific for 4 out of 5 clinical Pseudomonas aeruginosa isolates, minimally labeled non-Pseudomonas bacteria, and bound to both biofilm derived and planktonic Pseudomonas cells. The authors created chimeras and generated aptamers St17Lp21, St21Lp17. The chimeric aptamers showed improved binding and enhanced specificity for Pseudomonas bacteria as compared to the parent non-chimeric aptamers but were still unable to differentiate between biofilm and planktonic cells. This is not surprising since the biofilm-derived cells were washed and vortexed to release cells and remove alginate and exopolysaccharides. Mechanical stress induced by vortexing can destroy larger surface structures such as fimbriae and flagella. The lack of counter-selection coupled
with the vigorous washing steps may have exposed cell surface structures not unique to the biofilm-derived *Pseudomonas*. Using counter selection could have eliminated sequences that bind to surface structures such as LPS or ubiquitous OMPS that are common in both planktonic and biofilm-derived *Pseudomonas*.

Aptamers against *Yersinia enterocolitica* were generated using Cell-SELEX with bacteria grown at 26°C (Shoaib et al., 2020). After counter selecting with several bacterial pathogens, the three aptamers M1, M5, and M7 were isolated (Table 1). *Y. enterocolitica* grown at 37°C showed reduced binding by the aptamers compared to bacteria grown at 25°C. Presumably this aptamer is specific for a cell surface component mostly expressed at low temperature. This study illustrates another characteristic of bacteria, which are temperature dependent surface structure and morphological changes. In the case of *Y. enterocolitica* specifically, the bacterium inhibits flagellum synthesis at 37°C (Kapatral et al., 1996). Components of the LPS are also temperature regulated (Bialas et al., 2012).

**APTAMERS TARGETING VIABLE CELLS**

The ability to differentiate between dead and viable cells has important implications when assessing the risk or hazard of a microbe. For example, it would be costly and inefficient to administer shutdowns or disinfection protocols for the presence of dead pathogens in a system. The detection of viable cells is also important to determine the efficacy of water disinfection protocols. Some aptamers are able to differentiate between live and dead cells. Aptamer 33, specific for *Salmonella enterica* serovar Typhimurium, does not bind heat-killed cells (Table 1; Joshi et al., 2009). This aptamer might therefore be useful for monitoring the efficiency of heat-based disinfection. This aptamer is described in more detail below. Another example is aptamer ONS-23 created against whole cell *C. jejuni* (Table 1; Dwivedi et al., 2010). This aptamer was developed, using cell-SELEX, against a chicken isolate showing characteristic *C. jejuni* morphology (Thomas et al., 2002). Given that *C. jejuni* is found on raw poultry as well as in the gastrointestinal tract and feces of animals (Mughini-Gras et al., 2016), 20 bacterial species were used for counter selection, including food-borne pathogens, enteric bacteria, non-enteric bacteria and lactic acid bacteria. ONS-23 is therefore highly specific to *C. jejuni* strains showing minimal binding to non-*C. jejuni* strains (Dwivedi et al., 2010). Furthermore, ONS-23 does not bind non-viable *C. jejuni* (Kim Y. J. et al., 2018) indicating that it is specific for a surface structure only present on live *C. jejuni* cells (Kim Y. J. et al., 2018). Though this aptamer was not tested for water application, its selective properties for viable *C. jejuni* makes it promising for monitoring disinfection processes.

**APTAMERS TARGETING SOURCE-OR APPLICATION-SPECIFIC ISOLATES**

Isolates that are representative of the sample source of the downstream application should be used during aptamer development to ensure usefulness of the aptasensor. Aptamers E1, E2, and E10 were generated against a non-pathogenic *E. coli* strain of fecal origin (Crooks strain) using cell-SELEX (Table 1; Kim et al., 2013). For counter selection a combination of fecal coliform species and two Gram positives were used. The resulting aptamers were better at binding *E. coli* isolates of fecal origin than others and showed low binding to other species including laboratory strains of *E. coli* (Kim et al., 2013; Jin et al., 2017; Wu et al., 2017). A detection system using aptamer E2 was able to detect the Crooks strain in spiked tap water, pond water and milk, making it promising for *E. coli* detection in water (Jin et al., 2017).

**APTAMERS TARGETING SPECIFIC SURFACE STRUCTURES**

Surface structures can be differentially expressed in response to growth states and environment (Justice et al., 2004; Van Der Woude and Bäumler, 2004; Liu et al., 2012; Fonseca and Swanson, 2014; Li et al., 2015). If the aptamer surface target is not differentially regulated then aptamers may bind cells in several conditions, including exponential and post-exponential phase. Examples of these are the ST2P aptamer against whole cell *S. enterica* Typhimurium (Duan et al., 2013b, 2014, 2016) and the *E. coli* E2 aptamer (Kim et al., 2013; Jin et al., 2017; Wu et al., 2017). Instead of whole cells, surface structures related to virulence can also be used as aptamer targets. The pathotype EHEC (*E. coli* enterohemorrhagic) contains the infamous O157:H7 serotype which is strongly linked to deadly outbreaks from contaminated drinking water (Solomon et al., 2002; Ali, 2004; Saxena et al., 2015). For detecting this serotype, the specific variant of LPS can be exploited. *E. coli* aptamers a-aptamer and c-aptamer were created against LPS of *E. coli* O157:H7 (Table 1; Bruno et al., 2009). These aptamers were used in several aptasensing platforms to detect whole *E. coli* O157:H7 cells with great specificity, showing minimal signals with other serotypes (Wu et al., 2015; Díaz-Amaya et al., 2019a,b; Hao et al., 2019; Jiang et al., 2020). The aptamers could bind to heat-killed and formalin killed *E. coli* (Hao et al., 2019; Jiang et al., 2020). This is likely due to the fact that these treatments do not negatively affect the LPS (Gao et al., 2006; Chafin et al., 2013). This approach allowed for very specific aptamers to be developed; however, since the target persists after killing of cells, the aptamers are of limited use for monitoring the efficiency of disinfection programs in water. This illustrates the need for designing aptamers relevant to the downstream application.

Outer membrane proteins (OMP) of Typhimurium were used to create Aptermer 33. Counter selection was done with purified LPS of the *Salmonella* isolate as well as OMPS and LPS from *E. coli*. Aptermer 33 showed pan-serovar specificity, binding to seven different serovars of *S. enterica* in one study and four different *S. enterica* serovars in another study (Joshi et al., 2009; Hasan et al., 2018). The aptamer was used in a fluorescence aptasensor to detect whole Typhimurium in water samples from different sources highlighting its potential for detection in water (Duan et al., 2012). The aptamer does not bind to heat-killed...
Typhimurium which is to be expected as most OMPs are heat labile (Oh et al., 2017). The authors also observed that the aptamers could not bind S. enterica serovars Tennessee and Muenchen. This suggests that the aptamer may not have broad serovar specificity.

**DISCUSSION**

Aptamer-coupled biosensors are promising systems for the detection of pathogens in water samples but are limited in real-world applications. There are a few things to consider to improve aptamers practicality in aptasensing technology (Figure 1). Many studies do not explicitly report the growth states and conditions used during cell-SELEX or during subsequent testing of the aptamers (Table 1 and Supplementary Table 1). For example, OD_{600} values are meaningless without details about the growth conditions, including medium, temperature and aeration. We suggest that instead of reporting OD_{600}, the growth phase should be determined and reported, as done by Zou et al. (2018), as this would offer insight into an aptamer’s potential for specific applications. Regardless, it is important to keep the end goal in mind while developing aptamers. For example, monitoring efficiency of disinfection program will require discerning viable cells from dead cells. Aptamer ONS23 and Aptamer 33 are able to distinguish between live and dead cells (Joshi et al., 2009; Dwivedi et al., 2010; Oh et al., 2017; Kim Y. J. et al., 2018). A cell-SELEX strategy for such an application could use dead cells for counter selection. Another factor to consider is the physio-morphological state of microbes. This ensures that the microbial target possesses traits and characteristics that are representative of what’s typically found in the environment that will be sampled. For example, biofilm-derived cells might be used (Soundy and Day, 2017), but care must be taken not to remove the biofilm-specific target when preparing the target for cell-SELEX. Alternatively, if the end goal is to detect pathogens in water, then bacteria suspended in water may be used as the target (Saad et al., 2020). Lastly, it is not trivial to select appropriate strains for counter selection. This will impact aptamer affinities for targets in source environments. A possible approach is to use a cocktail of strains for the target species and a cocktail of species typically found in the same environment for counter-selection (Dwivedi et al., 2010; Kim et al., 2013). In conclusion, it is necessary to better elucidate the microbial target and the limitation of its cognate aptamer to help push microbial aptasensing platforms to market. As such a collaborative effort is needed between academics and stakeholders (governments, industry, engineers) to develop both transducer and aptamer technologies for specific microbial contaminants in the context of source water, taking into account the particularities of the microbe and its physiological state.

**AUTHOR CONTRIBUTIONS**

MS reviewed the literature and compiled the information reported here, and wrote the first draft of the manuscript. MS and SPF edited the manuscript. Both authors approved submission of the manuscript.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2021.643797/full#supplementary-material

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**Conflict of Interest:** The authors, together with Maryam Tabrizian (McGill University, Department of Biomedical Engineering), are the inventors of aptamers R10C1 and R10C5, subject of patent applications filed in United States, patent application number US 16/850,355; and in Canada – patent application number pending at the time of revised manuscript submission. At the time of submission of the manuscript, the applications were under review.

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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