The global need to improve bacterial detection in liquid media has motivated multidisciplinary research efforts toward developing new approaches that overcome the shortcomings of traditional techniques. We recently proposed the use of oligomers of acylated lysyls (OAKs) in their resin-linked form (ROAKs) for the efficient, robust, and inexpensive filtration of bacteria. Here, we investigate the potential for the use of ROAKs in downstream applications, we first examined the capacity of ROAKs to capture bacteria as a function of environmental conditions and structure–activity relationships (SARs). We next assessed their ability to release the captured bacteria and then combined both abilities to improve real-time PCR outcomes. ROAKs were able to deplete liquid samples of bacterial content after incubation or continuous flow, illustrating the efficient capture of different bacterial species under a wide range of ionic strength and pH conditions. We also show circumstances for the significant release of captured bacteria, live or dead, for further analysis. Finally, the SAR study revealed a shorter ROAK derivative exhibiting a capture capacity similar to that of the parent construct but the increased recovery of ROAK-bound bacteria, enabling improvement of the detection sensitivity by 20-fold. Collectively, the data support the potential usefulness of a simple, robust, and efficient approach for rapid capture/analysis of bacteria from tap water and, possibly, from more complex media.

To address the growing global need for improved rapid detection of pathogenic bacteria, various modern techniques have been developed to overcome the shortcomings of traditional microbiological and biochemical assays, including sensitivity, efficiency, and reliability (1–3). Alongside these advantages, however, modern tools, such as real-time PCR and immunological methods, also present limitations that may include complexity, requirement for prior knowledge, the limited ability of specific reagents to recognize new emerging pathogens, and/or development cost issues that prevent quick, on-site assays (4, 5). There is thus a clear need for improved tools that address these inherent limitations.

Analyses with antimicrobial peptides (AMPs) are among a few promising approaches that have been proposed for the multitargeted detection of bacteria, as AMPs offer broad-spectrum efficacy and have relatively simple chemical structures (6–8). These ubiquitous small molecules (9–11) are well-known for their activities against bacteria (12, 13), viruses (14), fungi (15), and protozoa (16). Consequently, AMPs have been considered a potential source for new therapeutics (17) but also for applications that exploit their intrinsic high affinity for microbes and, more specifically, for the microbial cell membrane(s) (18, 19). Although not fully understood, the interaction of AMPs with microbial membranes includes an initial strong electrostatic attraction step between the cationic peptide and negatively charged superficial microbial components, namely, the lipoteichoic acids (LTAs) of Gram-positive bacteria (20, 21) and lipopolysaccharides (LPSs) of Gram-negative bacteria (22, 23). While this interaction was extensively investigated and believed to lead to a host of cytotoxic mechanisms, AMPs were also suggested to be useful as recognition molecules for bacterial detection, both in vitro and in vivo, using radioactive or fluorescent labels (6, 24). Recent approaches have further attempted to exploit AMPs as capture molecules (25, 26), enabling multilayer detection, as opposed to target-specific detection in antibody-based assays (7). Another approach proposed the use of simple but robust synthetic constructs that mimic the structure and activity of AMPs for efficient bacterial capture in aqueous samples (27). The recent literature provides quite a rich report on various peptidomimetic strategies that well address some of the main drawbacks of AMPs, including protease sensitivity and production costs (28, 29), with their design being inspired from the structural and chemophysical attributes of natural AMPs (i.e., structure, charge, hydrophobicity, and amphipathic organization) (30, 31).

Oligomers of acylated lysyls (OAKs) represent a family of such synthetic mimics of antimicrobial peptides which exhibit a high affinity for the surface components of various bacteria (32, 33). The study that initially investigated the immobilization of an OAK on a polystyrene resin and its subsequent use for bacterial capture (depicted in Fig. 1) reported the ability of such a resin-linked OAK (ROAK) to sequester a broad spectrum of bacterial species (27). That study focused on a particular ROAK, constructed with the sequence K-7α12 (where α12 represents an aminododecanoyl-lysyl subunit) (Table 1), whose capture activity was suggested to depend on both charge and hydrophobicity attributes. The rapid and efficient bacterial capture of both Gram-negative bacteria (Escherichia coli, Vibrio cholera) and Gram-positive bacteria (Staphylococcus aureus, Enterococcus faecalis) by that ROAK was...
demonstrated. Hence, available preliminary data suggest that the ROAK approach might be useful for the separation/concentration of microbial cells from large volumes of samples, thereby providing advantages over current methods, such as robustness, simplicity, and usefulness, as a device to capture a broad spectrum of microbes (1).

The aim of this study was to verify such a potential. Thus, after validating the aforementioned preliminary observations under stringent conditions, the investigation was extended to include a structure-activity relationship (SAR) study with the aim of improving the system’s attributes, including the release of the captured bacteria for potential downstream applications, such as a concentration method to enhance the sensitivity of bacterial detection.

MATERIALS AND METHODS

ROAK preparation. ROAKs were synthesized by the solid-phase method (34) by using 4-methylbenzhydrylamine resin (Sigma-Aldrich) and applying N-(9-fluorenyl)methoxy-carbonyl (Fmoc) active ester chemistry on a 433A peptide synthesizer (Applied Biosystems). OAK sequences obtained after cleavage of an aliquot were verified postsynthesis, followed by ultraperformance liquid chromatography-mass spectrometry analysis (Xevo G2 Tof/Acquity; Waters). ROAK deprotection was achieved by incubation in dichloromethane-trifluoroacetic acid (50:50) for up to 15 min at room temperature, after which the resin was washed twice with dichloromethane and twice with ethanol, lyophilized for 3 h, and stored at −20°C. Prior to use, the ROAK beads were routinely washed with ethanol (70%) and saline (0.85% NaCl).

Bacteria. The bacteria tested were Escherichia coli ATCC 35218, Pseudomonas aeruginosa ATCC 27853, and Klebsiella pneumoniae CI 1287. The bacteria were grown aerobically in Luria-Bertani (LB) broth at 37°C and then diluted to 10^8 into fresh LB broth and incubated under the same conditions described above for 2 h, after which the mid-log-phase cultures were diluted to 10^8 into fresh LB broth and incubated under the same conditions described above for 2 h, after which the mid-log-phase cultures were diluted to 10^8 CFU per ml (on the basis of the optical density measurement at 600 nm) and then diluted again to the concentrations specified below for each assay.

Capture assay. Bacterial capture was determined essentially as described previously (27), except that the spin columns (VectaSpin Micro; Whatman), which are no longer available commercially, were replaced by comparable ones (0.9-ml Pierce spin columns with a 10-μm-cutoff membrane; Thermo Fisher Scientific Inc.). Briefly, bacteria were incubated in 500 μl saline in a spin column containing analytically weighed ROAK beads (3 to 4 mg) or uncoated beads as a control. After 15 min incubation with shaking at 37°C, the columns were centrifuged (1 min at 5,000 × g) to separate the beads from the filtrate (medium containing the unbound bacteria). The capture efficacy was defined as the ratio of the bacterial count in a sample filtrate to that in its control experiment (i.e., using uncoated beads); thus, the capture percentage was calculated as 100 − [(f_CU) × 100], where f_CU and f are the bacterial counts of the ROAK and control filtrates, respectively. Bacterial counts were routinely achieved by plating of serial 10-fold sample dilutions for determination of the number of CFU after overnight incubation at 37°C. Alternatively, quantitative PCR (qPCR) was also performed on the E. coli samples, as detailed below.

To test for environmental effects, the bacteria were suspended in different media, as specified below; all salt solutions were filtered (0.2-μm-pore-size filters) prior to use; the effect of pH was assessed using phosphate-buffered saline (PBS) adjusted with 1 N HCl or NaOH; human blood samples for research were acquired from the Israeli Blood Bank.

To recover the ROAK-bound bacteria, the beads were spin washed in saline, after which an elution agent (as specified below) was added, and the mixture was vortexed for 2 to 3 s and then centrifuged at 5,000 × g for 1 min. Aliquots of each sample were plated for determination of the number of CFU, and the bacteria were concurrently quantified by qPCR, as detailed further below. The eluate was assessed for the percentage of bacteria that eluted from the bound bacteria, calculated as (E/(f_CU − f)) × 100, where E is the bacterial quantity in the eluted sample and f_CU − f is the quantity of ROAK-bound bacteria.

ROAK columns. Continuous-flow column filtration was performed essentially as described previously (27). Briefly, ROAK beads (10 mg) were packed in a glass pipette (restrained by glass fibers) and preconditioned in saline. Inocula consisting of saline spiked with 10^6 CFU of E. coli ATCC 35218 were passed through the column at a flow rate of 2.5 ml/min using a peristaltic pump (0.05 to 10 ml/min; BT50-1; Baoding Longer Precision Pump, China). For bacterial release, the column was rinsed with 1 ml saline and then eluted with 1 ml 0.5 M CaCl_2 solution. Each inoculum, filtrate, saline wash, and elution fraction was analyzed for bacterial quantification by qPCR and determination of the numbers of CFU.

Bacterial quantification by qPCR. Genomic DNA was extracted from aliquots of 0.3 ml of each sample by ethanol precipitation. Samples were centrifuged at 10,000 × g (5 min), the supernatant was removed, and the pellet was resuspended in 1 ml of ethanol-water (70:30), followed by addition of 33 μl of 3 M Na acetate, incubation for at least 1 min in liquid nitrogen, and centrifugation at 16,000 × g (16 min) and 4°C. The supernatant was discarded, the pellet was suspended in 20 μl of 0.1X TE buffer (1 mM Tris in 0.1 mM EDTA, pH 8.0), and the DNA was submitted to qPCR analysis, as follows: qPCRs were carried out in MicroAmp Fast optical 96-well reaction plates using a StepOnePlus real-time PCR system (v.2.0; Applied Biosystems) with a reaction volume of 10 μl that was composed of 5 μl Fast SYBR green master mix (Applied Biosystems), 0.8 μl of each of the forward and reverse primers, and 3.4 μl of DNA sample.

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Designation</th>
<th>Capture efficacy</th>
<th>(%)</th>
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<tbody>
<tr>
<td>K-NGC12-K-NGC12-K-NC12-K-NGC12-K</td>
<td>95 ± 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K-NGC12-K-NC12-K-NC12-K</td>
<td>90 ± 1</td>
<td></td>
<td></td>
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<tr>
<td>K-NC12-K-NC12-K-NC12-K</td>
<td>40 ± 15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K-NC12-K-NC12-K</td>
<td>86 ± 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K-NC12-K-NC12-K</td>
<td>5 ± 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K-NC12-K</td>
<td>6 ± 5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| a | K, lysyl residue; NC12, aminododecanoyl residue; α12, aminododecanoyl-lysyl subunit. |
| b | The reported capture efficacy is a typical outcome representing the mean value of three independent experiments and is expressed as a percentage of the value for the control (using uncoated beads), determined with the E. coli capture assay (defined in the Materials and Methods section) using CFU enumeration. BDL, below the detection limit. |
The thermal cycling conditions were as follows: amplification started with a step of enzyme activation and initial denaturation at 95°C for 2 min, followed by 40 cycles consisting of denaturation at 95°C for 10 s and annealing and extension at 60°C for 15 s. A nontemplate control (NTC) and a positive control were used to validate each PCR. PCR data were generated and analyzed using StepOne software (Applied Biosystems).

Bacteria were quantified by comparing the threshold cycle (the number of cycles at which the fluorescence exceeds the threshold) of each sample to a standard curve composed of the threshold cycles of bacterial samples at serial 10-fold dilutions of bacterial suspensions in saline which were concomitantly subjected to the same DNA extraction method. Note that qPCR was performed only for samples containing *E. coli*, using primers specific for the *dxs* gene, a single-copy gene for deoxy-xylulose-phosphate synthase (35). The primer sequences were 5′-CGAGAAACTGGCGAT CCTA-3′ for the forward primer and 5′-CTTCATCAAGCGGTTTCAC A-3′ for the reverse primer, and the PCR product length was 113 bp. Primers, purchased from Sigma-Aldrich Co., were diluted to 2.5 μM in 0.1X TE buffer according to the manufacturer’s instructions.

**Statistical analysis.** Data represent the means and standard deviations calculated from at least two independent experiments and were analyzed using a one- or two-tailed unpaired t test with an assumption of equal variance.

**RESULTS AND DISCUSSION**

The study initially focused on assessing the ability of the reference ROAK (i.e., composed of the sequence K-7α12) to capture bacteria after a period of incubation under different conditions, as summarized in Fig. 2. Figure 2A shows the high efficacy (81 to 100%) of capture of three representative and medically relevant bacteria using different inocula ranging from 10⁴ to 10⁶ CFU/ml. Bacterial quantification was achieved both by determination of the number of CFU (white columns) and by qPCR (gray columns). DDW, doubly distilled water. *P < 0.05 by one-tailed t test compared to bacterial capture in doubly distilled water. (D) Representative data comparing bacterial capture in saline and human whole blood spiked with 10⁶ CFU/ml bacteria. Symbols for the bacteria are as defined in the legend to panel A. Presented are the average percentages of bound bacteria compared to the amount of bound bacteria for the control (using uncoated beads). *, P < 0.05 by two-tailed t test. Data were obtained from at least 2 independent experiments. Error bars represent standard deviations.
higher than those obtained in the assessment based on CFU counts, a discrepancy that may stem from bacterial death at the high concentrations.

Thus, both sets of data (Fig. 2A and B) are consistent with previous findings (27), arguing for a high-affinity interaction between bacteria and ROAK beads, an interaction where electrostatic attraction is likely to play a major role when taking place in an aqueous environment. This notion is consolidated by additional experimental data that tested the effect of pH values ranging from 3 to 9 in PBS, where a sensibly similar capture profile was obtained (Fig. 2C); i.e., no significant interference with E. coli capture at pH 3 to 9 compared with that at pH 7 was detected. Another line of evidence is apparent from the data shown in Fig. 2D, which reveal some bacterial capture in whole blood (up to 23%), suggesting that bacterial capture may, in principle, take place even in extremely complex media. In fact, preliminary experiments performed under conditions comparable to those described above showed that a 10-fold dilution of whole blood leads to a significant increase (up to about 50%) in bacterial capture (data not shown). Future studies might investigate this aspect, which seems to have clinical significance.

Collectively, these data indicate that the ROAK system may be most useful for bacterial capture in water and possibly in more complex media as well, although such capture can be significantly masked/inhibited, namely, by high levels of salts and plasma components.

Next, we attempted to better understand the structure-activity relationships (SARs) of the ROAK system, aiming to establish the minimal requirements for effective bacterial capture. For this purpose, we compared the capture efficacies of different OAK derivatives generated by decreasing the number of α12 subunits from 7 to 1 or by altering the N-terminal residue (the free end) of selected OAKs. A representative set of data obtained from this SAR study using E. coli is shown in Table 1. The data essentially revealed that efficient bacterial capture can be maintained in several ROAKs composed of sequences shorter than K-7α12 (e.g., K-5α12 or K-4α12), whereas only sequences shorter than K-3α12 exhibited insignificant capture activity.

The data listed in Table 1 also showed that omission of the N-terminal lysine in the shorter sequences (i.e., short sequences starting with an aminododecanoyl instead of a lysine) typically resulted in a significant loss of capture capacity (compare, for instance, the results for the pair K-4α12 and 4α12 or for the pair K-3α12, and 3α12), suggesting a functional (or, possibly, compensating) role for a multiply charged moiety (+2) at the N-terminal position of short OAKs.

To gain further insight from the SAR study, we next investigated the effect of incubation time on the capture capacity. As the reference OAK is known for its rapid capture kinetics (27), we tested capture after only brief incubation periods. The data shown in Fig. 3A revealed practically no change in the number of captured E. coli bacteria for samples assessed after 5 and 10 min incubation, whereas from analysis after 1 min incubation, a positive correlation between the capture kinetics and ROAKs displaying a high capture capacity was evident (Table 1). In this respect, K-3α12 was the shortest sequence to best combine both properties, as observed when using E. coli. Note, however, that this correlation was also observed for other bacterial species (data not shown); i.e., shorter ROAKs generally maintained similar capture kinetics when E. coli was replaced with P. aeruginosa or K. pneumoniae, including at other (e.g., 10-fold lower) inocula.

Figure 3B compares the capture capacity of the reference ROAK with the capture capacities of its shorter derivatives and highlights the changes in hydrophobicity of the free sequences in each ROAK, expressed in terms of the percent acetonitrile (ACN) required for elution from a C18 column using reversed-phase HPLC at room temperature, are also shown. Data were obtained from at least 2 independent experiments. Error bars represent standard deviations.

**FIG 3** Bacterial capture by ROAK derivatives. (A) Shown are the results of a representative experiment comparing the capture of E. coli (5.1 ± 0.2 log CFU in 0.5 ml saline) after different incubation periods using ROAKs composed of K-7α12 (diamonds), K-5α12 (triangles), K-4α12 (inverted triangles), K-3α12 (circles), and K-2α12 (squares). (B) Capture efficacy of K-7α12 ROAK after 15 min incubation with E. coli (white bars), K. pneumoniae (bars with horizontal stripes), and P. aeruginosa (bars with vertical stripes). *, P < 0.05 by one-tailed t test compared to K-3α12; **, P < 0.01 by one-tailed t test compared to K-3α12. The changes in hydrophobicity of the sequences in each ROAK, expressed in terms of the percent acetonitrile (ACN) required for elution from a C18 column using reversed-phase HPLC at room temperature, are also shown. Data were obtained from at least 2 independent experiments. Error bars represent standard deviations.
Another interesting observation from the SARs relates to the fact that K-3α12 exhibited a lower capture efficacy with *P. aeruginosa* than with other bacteria, as can be seen in Fig. 3B. On the other hand, the K-5α12 analog clearly displayed a capture efficacy for *Pseudomonas* superior to that of the reference ROAK, K-7α12, which otherwise seems to be the most potent for the capture of other bacteria. These discrepancies might hint that specific sets of characteristics (e.g., optimal charge and hydrophobicity combinations) in the OAK sequence are required for the optimal capture of specific bacterial species, to account for their specific surface topographies.

To further characterize the capture attributes, we next challenged the bond strength by exposing ROAK-bound bacteria to various washes in order to investigate their ability to act as eluting agents, as summarized in Fig. 4. Figure 4A compares the elution efficiencies of ethanol and salt solutions using *E. coli* captured by K-7α12 ROAK beads. Note that, as observed in Fig. 2B, the data from both quantification methods were generally coherent, although somewhat smaller quantities (up to 8%) were observed by determination of the numbers of CFU (data not shown), likely reflecting the antibacterial effect of salts. As shown in Fig. 4A, recovery from ethanol washes was typically less than 1% of the bound bacteria, whereas salt solutions that inhibited bacterial capture (refer to Fig. 2B) displayed a considerably higher elution power (up to about 5, 12, and 17% for NaCl, MgCl2, and CaCl2, respectively).

Figure 4B compares the elution yields of the reference ROAK and its shorter derivative, K-3α12, when similarly washed with 70% ethanol or 0.5 M CaCl2. The data revealed that, unlike K-7α12, which allowed recovery of free bacteria only upon the CaCl2 wash, the shorter construct, which was less hydrophobic and less charged, was able to free over 10% of the bound bacteria when either one of these eluents was used, thereby validating the notion of a weaker interaction between *E. coli* and K-3α12.

Collectively, these findings provide coherent evidence arguing for affinity interactions between ROAKs and Gram-negative bacteria and that despite the high binding affinity, a significant portion of the captured bacteria (live or dead, using salt or ethanol, respectively) can be recovered. Based on these data, we hypothesized that the combination of both capabilities (i.e., capture and release) might be beneficial for active filtration of bacterial samples and, potentially, for enrichment of samples with low bacterial counts. In other words, the sensitivity of a detection method such as qPCR might be enhanced by harvesting the ability of ROAK to enrich the number of bacteria available in samples with low counts, namely, by exploiting the potential for efficient capture/concentration of bacteria from samples with large volumes, using a continuous-flow column filtration system like the one described previously (27).

To verify this hypothesis, we utilized a volumetric 1-ml glass pipette where ROAK beads (held between glass fibers) were put to use to filter saline inocula spiked with a constant number of *E. coli* bacteria (6.0 ± 0.5 log CFU), as summarized in Fig. 5. Figure 5A shows that both the K-7α12 and K-3α12 ROAKs maintained a high capacity for bacterial capture under these continuous-flow conditions, albeit the capacity was somewhat less for K-3α12 (i.e., 92 and 80% of their respective inocula), possibly due to the combined lower affinity and the shorter interaction time involved in the flow conditions. Note that this could be predicted from the kinetic profile shown in Fig. 3A.

![Figure 4](http://aem.asm.org/)

**FIG 4** Recovery of ROAK-bound *E. coli*. (A) Elution efficacies achieved with a wash step using ethanol (EtOH; 70%, vol/vol) or different salt solutions, expressed as a percentage of the bacteria bound to K-7α12, ROAK. For all salt solutions, *P* was <0.05 by a one-tailed *t* test compared to 0.1 M NaCl. (B) Elution efficacies achieved by 0.5 M CaCl2 (gray bars) and ethanol (bars with stripes) when the reference ROAK, K-7α12, was compared with its shorter analog, K-3α12. *, *P* < 0.05 by two-tailed *t* test comparing ethanol elution for the two ROAKs. The quantities in both panels were obtained by the qPCR method. Data were obtained from at least 2 independent experiments. Error bars represent standard deviations.

![Figure 5](http://aem.asm.org/)

**FIG 5** Bacterial concentration after continuous-flow filtration. Inocula spiked with *E. coli* ATCC 35218 (6.0 ± 0.5 log CFU) were passed through a glass column (a 1-ml pipette) containing 10 mg of the specified ROAK at a flow rate of 2.5 ml/min, followed by a saline wash. (A) Capture efficacy determined after passing a 50-ml sample and comparing the bacterial counts in the filtrate versus those in the inoculum. (B) Elution of the bound bacteria from the assay whose results are presented in panel A was achieved with a 1-ml CaCl2 (0.5 M) wash and was determined as the ratio of the amount of eluted bacteria to the amount of column-bound bacteria. Statistical analysis was done by a one-tailed *t* test. (C) Bacterial concentration factors calculated for the specified inoculum volumes as the ratio of the eluate count per milliliter to the inoculum count per milliliter. *, *P* < 0.05 by a two-tailed *t* test compared to the inoculum concentration. All bacterial quantifications were obtained by qPCR. Data were obtained from at least 2 independent experiments. Error bars represent standard deviations.
Most significantly, however, the elution yield from the K-3\(\alpha\)12 column was substantially higher than that from the K-7\(\alpha\)12 column; i.e., the elution yield from the K-3\(\alpha\)12 column represented a 5-fold increase compared to that from the K-7\(\alpha\)12 column (Fig. 5B). Thus, unlike elution attempts following the capture assay under incubation conditions which typically resulted in a 15 to 20% recovery of captured bacteria from a CaCl\(_2\) wash (Fig. 4B), here, under the flow conditions used, the K-7\(\alpha\)12 column was dis- inclined to release the bound bacteria and only the K-3\(\alpha\)12 column maintained a similarly high recovery, i.e., about 20% of the bound bacteria. Again, this might reflect differences in binding affinities between E. coli and these OAKs. One might imagine a repetitive process in which multiple adhesion and dissociation steps occur between juxtaposed ROAK beads all along the column height. Consequently, it would be less difficult to elute from the K-3\(\alpha\)12 column than from the K-7\(\alpha\)12 column, where the binding affinity is apparently somewhat higher, as evidenced quantitatively and kinetically in Fig. 3.

Notwithstanding these findings, the data distinctly argue that the K-3\(\alpha\)12 ROAK column assay represents a rapid bacterial enrichment procedure since bacterial counts could be elevated by about 7-fold (referred to as a “concentration factor”). Because capture efficacy is consistently high, this concentration factor is affected only by elution efficiency. Thus, one might expect that using the ROAK column assay for samples with higher volumes will further enhance the concentration factor, as the elution yield is not a function of the initial bacterial count per ml but of the total number of bacteria bound to ROAK beads. Figure 5C shows the trend line supporting this claim. Thus, different sample volumes with identical inoculum values were applied to the ROAK column, testing the concentration factor (i.e., the ratio of the bacterial count in the eluate to that in the inoculum). By applying a higher inoculum volume (100 ml), often required in standard tests (36, 37), the concentration factor was increased to about 20-fold, a number that, at least theoretically, should further increase with increasing sample volumes.

Noteworthy is the fact that these values, which are based on qPCR analysis, are almost 2-fold higher than those obtained following determination of the number of CFU (data not shown); i.e., similar to previous analyses, bacterial quantification based on CFU counts exhibited a similar trend line but lower concentration factors. From the difference between these values, we conclude that a significant proportion (54% ± 17%) of the eluted bacteria was viable.

In conclusion, the current work extends the initial ROAK approach previously described (27) by improving our understanding of the OAK-bacterium interaction both qualitatively and quantitatively and by providing a SAR perspective as well as evidence for potential implementation of the ROAK system. Namely, the data provide evidence of the ability of ROAK columns to deplete a sample of bacteria using high-affinity OAKs (e.g., K-7\(\alpha\)\(_{12}\)) or, alternatively, to improve the sensitivity of qPCR-based bacterial detection by using lower-affinity OAKs (e.g., K-3\(\alpha\)\(_{12}\)). Thus, in addition to its compositional simplicity and robustness, the new attributes highlight a potential advantage of the OAK approach over approaches that use antibodies (5) or AMPs (8), including in terms of how environmental conditions (pH, ionic strength, and complexity) might affect their performances.

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REFERENCES


