Integrating Phage Therapy into Endodontics for Increased Operational Readiness: Biofilm Reduction and Phage-Sealer Compatibility

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DISCLAIMER

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- *NMR&D Mission Statement: Navy Medicine Research & Development, with its insight into challenges faced by Sailors and Marines, is uniquely qualified to lead U.S. Navy research efforts to ensure healthy, fit, and lethal service members.*

DISCLOSURE / CONFLICT OF INTEREST

- *The author has no financial relationships or conflicts of interest to disclose.*
- *The author has no commercial associations, interests, or relationships that would pose a conflict of interest in connection with this presentation.*

Introduction & Clinical Significance

The Operational Challenge in Austere Environments

- High Dental Casualty Rates: Approximately 15% of active-duty service members experience dental emergencies annually.
- Operational Impact: Root canal treatment (RCT) complications account for 20.7% of dental emergencies in deployed environments.
- The Consequences: Failed RCTs cause severe pain, systemic infection, facial space loss, and compromise warfighter combat effectiveness.
- Financial Burden: Treatment costs range from $500 to $1,600 per case.
- The Primary Culprit: *Enterococcus faecalis* (*E. faecalis*) is the predominant pathogen isolated from failed RCTs and persistent endodontic infections.

Research Objectives

Integrating Phage Therapy into Root Canal Procedures

The Core Hypothesis: Can lytic bacteriophages targeting *E. faecalis* be utilized within endodontic treatments to prevent or eradicate persistent secondary infections?

Phase 1: Isolate and genetically/proteomically characterize novel bacteriophages targeting *E. faecalis*.

Phase 2: Evaluate the efficacy of isolated phages in reducing mature *E. faecalis* biofilms.

Phase 3: Assess the stability, viability, and compatibility of phages when integrated with common commercial endodontic sealers.

METHODS & OUTLINE

Phage Isolation

Sewage from Austin, Arlington, and Laredo, Texas mixed with *E. faecalis* culture in tryptic soy broth overnight. Clarified lysate was then serially diluted and plated in a top agar overlay. Plaques were picked and plated three times to ensure purity.

DNA Sequencing and Annotation

CD Genomics performed Illumina sequencing and auto-annotated the genome using Phage Commander. We manually corrected annotation using GeneMark, BLAST, and DNA Master.

Transmission Electron Microscopy (TEM) Imaging

Performed by University of Maryland, Baltimore County

Host Range

A total of 21 *E. faecalis* and *Enterococcus faecium* strains were mixed into top agar and plated;

5 µl of $1x10^8$ CFU/ml lysate were spotted onto plates and clearing was observed the next day

Growth Curves & Optical Density (OD_{600})

$1.1x10^6$ CFU of bacteria was mixed with several concentrations of phage from $1.1x10^7$ to $1.1x10^1$ PFU by 10-fold dilution and grown for 21 hours at 37º C in a 96-well plate, OD_{600} measured every 30 minutes in a shaking plate reader

Growth Curves & Endpoint CFU

Samples taken from wells of growth curve plate, serially diluted, and spotted on plates to quantify viable bacteria remaining after phage treatment

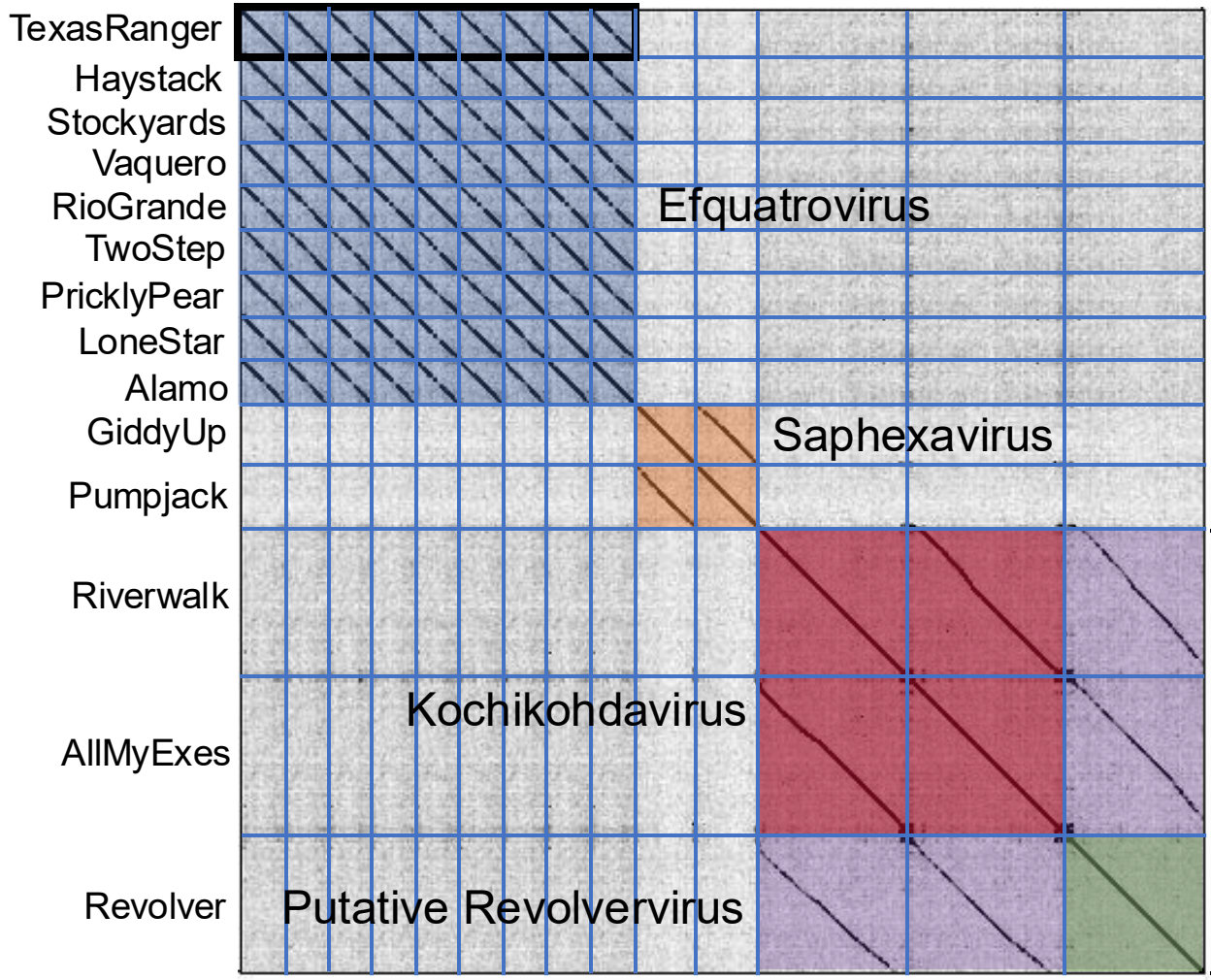
Biofilm Disruption

Orally derived commercially available *E. faecalis* 4082 and OG1RF were grown in 24-well plates, 1 ml/well, for two weeks at 37º C, no shaking. At the two-week mark 10^7, 10^5, or 10^3 PFU of phage were added to the wells and incubated for one week. Crystal violet staining was utilized

RESULTS

Taxonomy and Annotation

- Fourteen phage isolated
  - TexasRanger, Alamo, Haystack, TwoStep, PricklyPear, LoneStar, Stockyards, RioGrande, GiddyUp, Pumpjack, Vaquero, Riverwalk, AllMyExes, Revolver
- Taxonomically classified into three established viral genera (according to International Committee on Taxonomy of Viruses) and one potentially forming a new genus (Revolvervirus)



Dot Plot: Compares genomes nucleotide by nucleotide along both axes. Dark line represents high similarity, light or gaps in lines show dissimilarity. Blue lines used to separate genomes.

EFQUATROVIRUS

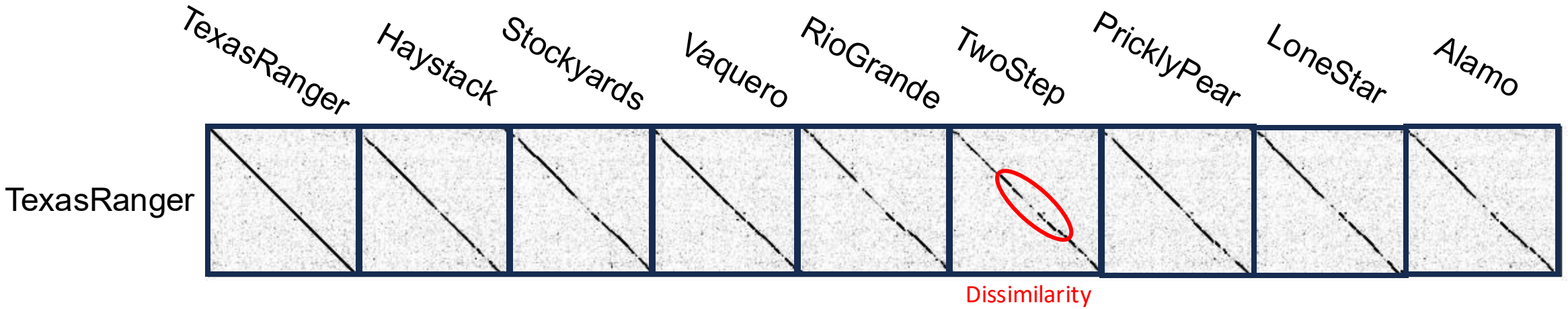


Table 1. Phage Morphology Measurements

	AllMyExes	Riverwalk	Revolver	Alamo	LoneStar	TwoStep	Vaquero	PricklyPear	TexasRanger	Haystack	RioGrande	Stockyards	Pumpjack	GiddyUp
Head Length	88 nm	94 nm	88 nm	53 nm	55 nm	54 nm	55 nm	55 nm	57 nm	55 nm	51 nm	55 nm	107 nm	107 nm
Head Width	79 nm	83 nm	79 nm	53 nm	50 nm	54 nm	52 nm	52 nm	51 nm	51 nm	49 nm	55 nm	42 nm	42 nm
Tail Length	170 nm	174 nm	170 nm	201 nm	200 nm	205 nm	206 nm	203 nm	201 nm	203 nm	200 nm	212 nm	132 nm	138 nm
Tail Width	20 nm	21 nm	19 nm	12 nm	11 nm	13 nm	12 nm	11 nm	12 nm	13 nm	10 nm	10 nm	10 nm	13 nm

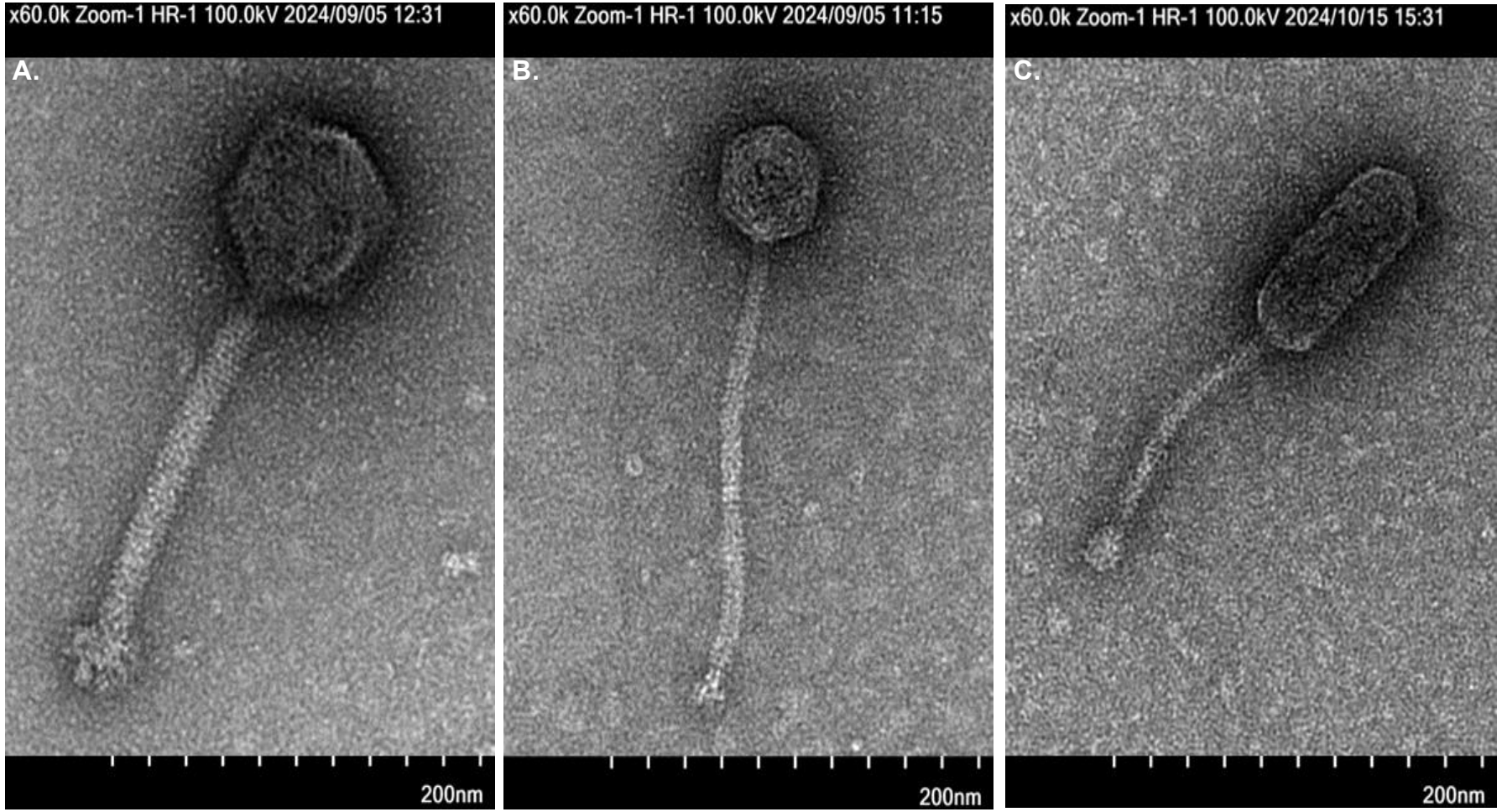


Figure 1.1 Transmission Electron Microscopy Images of (A) Revolver, (B) Alamo, and (C) GiddyUp.

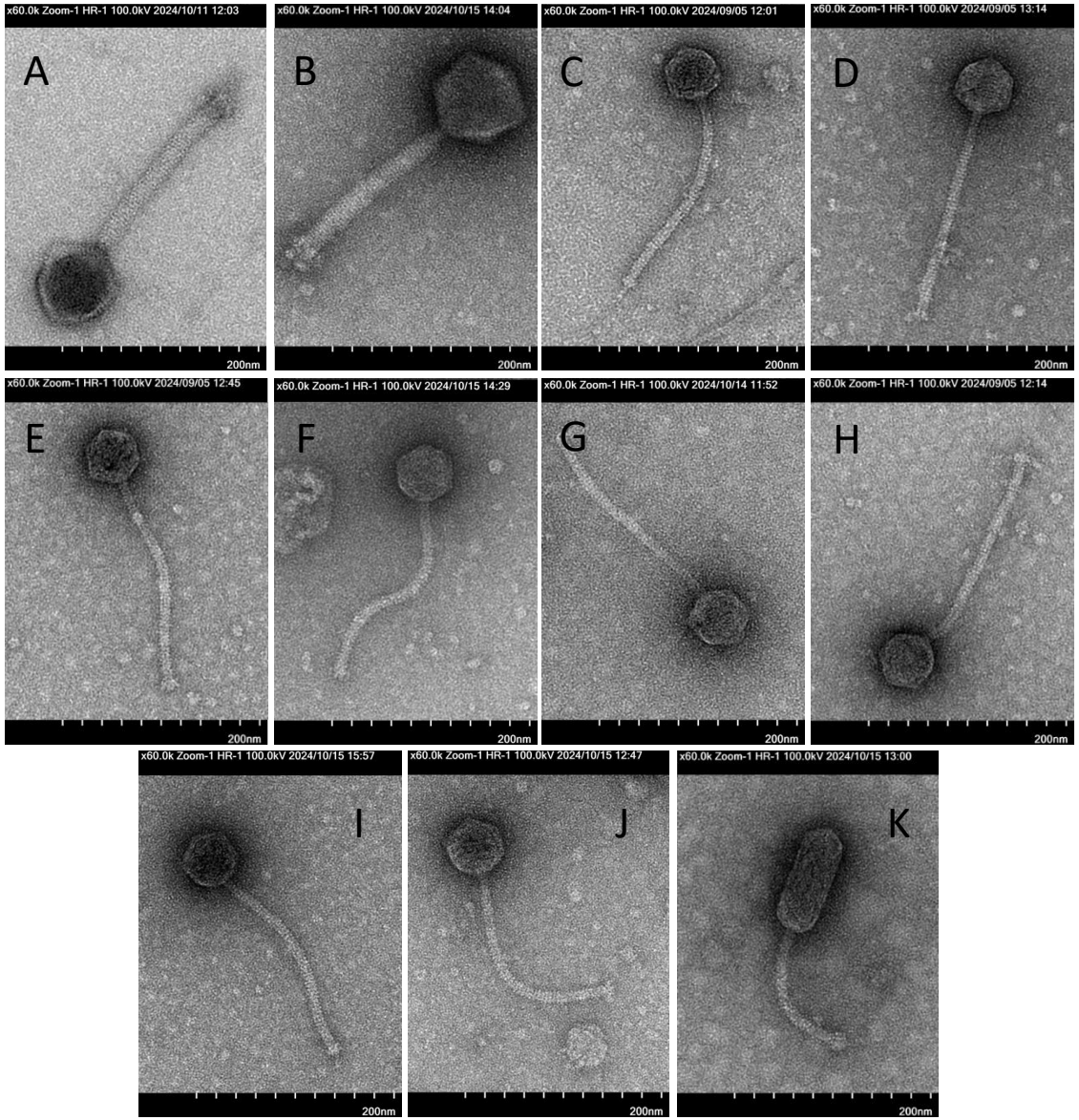


Figure 1.2 Transmission Electron Microscopy Images of (A) AllMyExes, (B) Riverwalk, (C) LoneStar, (D) TwoStep, (E) Vaquero, (F) PricklyPear, (G) TexasRanger, (H) Haystack, (I) RioGrande, (J) Stockyards, (K) Pumpjack.

Table 2. Phage Host Range

Bacteria\Phage	AllMyExes	Riverwalk	Revolver	Alamo	LoneStar	TwoStep	Vaquero	PricklyPear	TexasRanger	Haystack	RioGrande	Stockyards	Pumpjack	GiddyUp
4082	+	+	+					+	+	+	+	+		
OG1RF	+	+	+										+	+
29212		+		+	+	+							+	+
33186	+	+	+				+	+	+	+			+	
19434			+					+	+	+				
51299	+	+	+					+		+	+	+		
49624			+					+						
35667			+					+	+	+				
19433	+	+	+					+	+	+	+	+		
VRE01		+	+					+	+	+	+	+		
VRE02	+	+	+					+	+		+	+		
VRE05		+	+					+	+	+	+	+		
VRE11	+	+	+					+	+	+				
VRE20	+	+	+					+			+	+		
VRE29	+	+	+											
VRE6	+	+	+					+			+	+		
VRE13		+	+							+				
VRE19		+	+							+		+		
VRE24	+	+	+					+		+	+	+		
VRE25		+	+								+	+	+	
VRE30	+	+	+										+	
# of Strains Infected	12/21	18/21	20/21	1/21	1/21	1/21	1/21	14/21	9/21	12/21	10/21	11/21	5/21	2/21

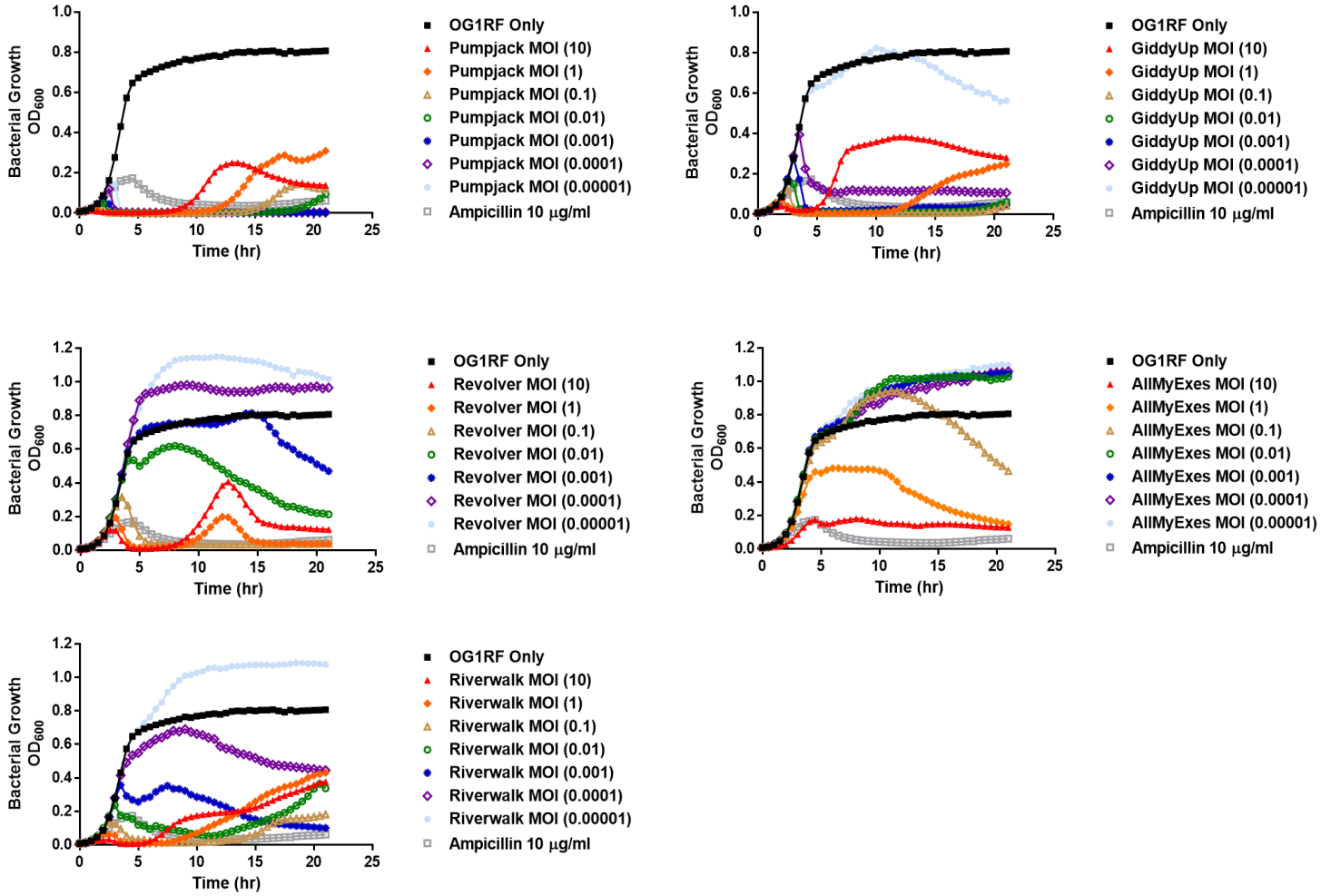


Figure 2. Phage Mediated Growth Curves of OG1RF. A total of 1.1×10^6 CFUs of bacteria were mixed with several amounts of phage ranging from 1.1×10^7 PFU to 11 PFU making multiplicities of infection (MOI) 10-0.00001. OD_{600} was measured over 21 hr. Five phage were tested as follows (A) Pumpjack (n = 3), (B) GiddyUp (n = 3), (C) Revolver (n = 3), (D) AllMyExes (n = 3), and (E) Riverwalk (n = 3). The same bacteria only (n = 11) and ampicillin (n = 11) controls are shown in all five graphs.

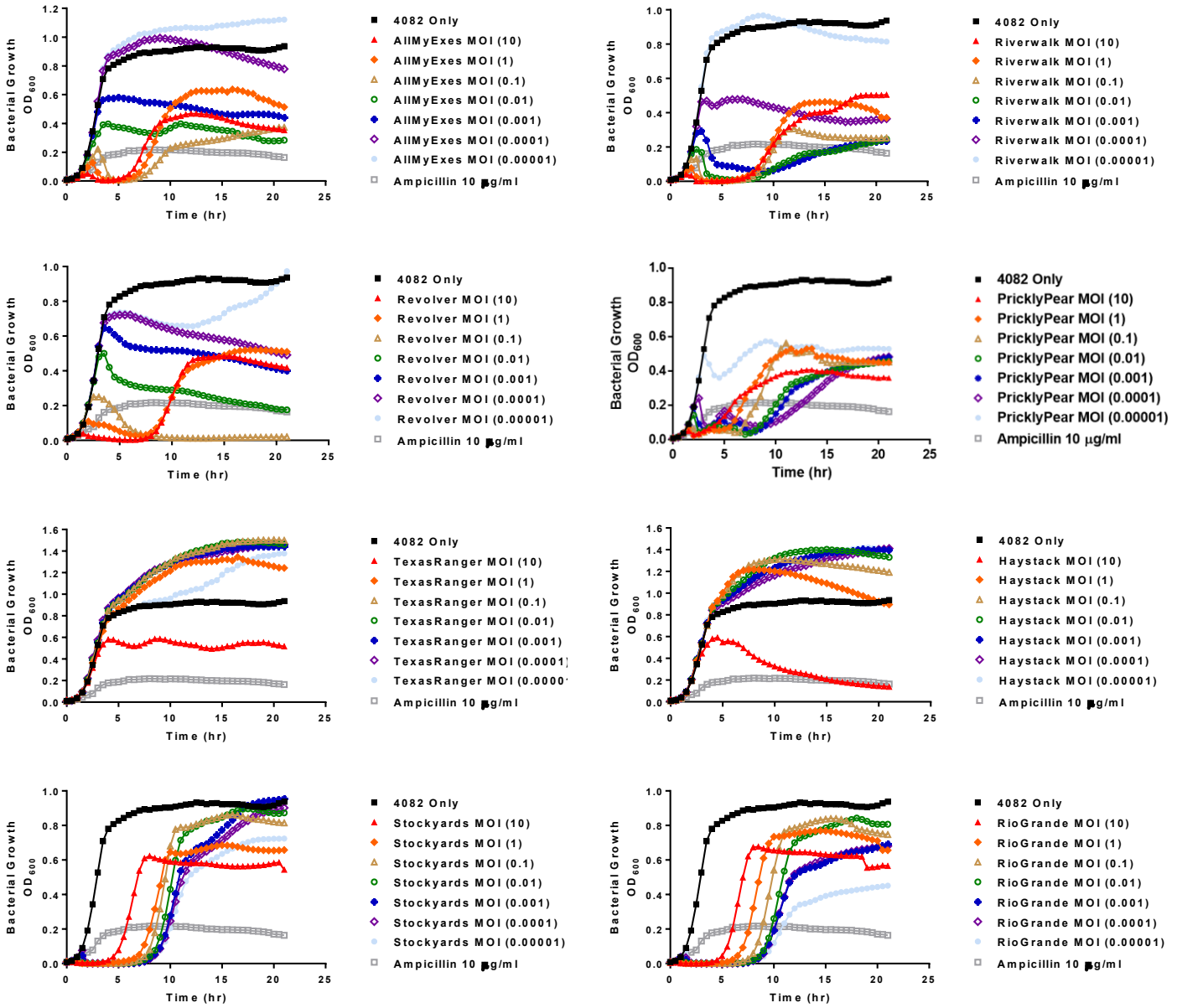


Figure 3. Phage Mediated Growth Curves of 4082. A total of 1.1×10^6 CFUs of bacteria were mixed with several amounts of phage ranging from 1.1×10^7 PFU to 11 PFU making multiplicities of infection (MOI) 10-0.00001. OD_{600} was measured over 21 hr. Eight phage were tested as follows (A) AllMyExes, (B) Riverwalk, (C) Revolver, (D) PricklyPear, and (E) TexasRanger, (F) Haystack, (G) Stockyards, and (H) RioGrande, n = 3 for all phage. The same bacteria only (n = 9) and ampicillin (n = 9) controls are shown in all eight graphs.

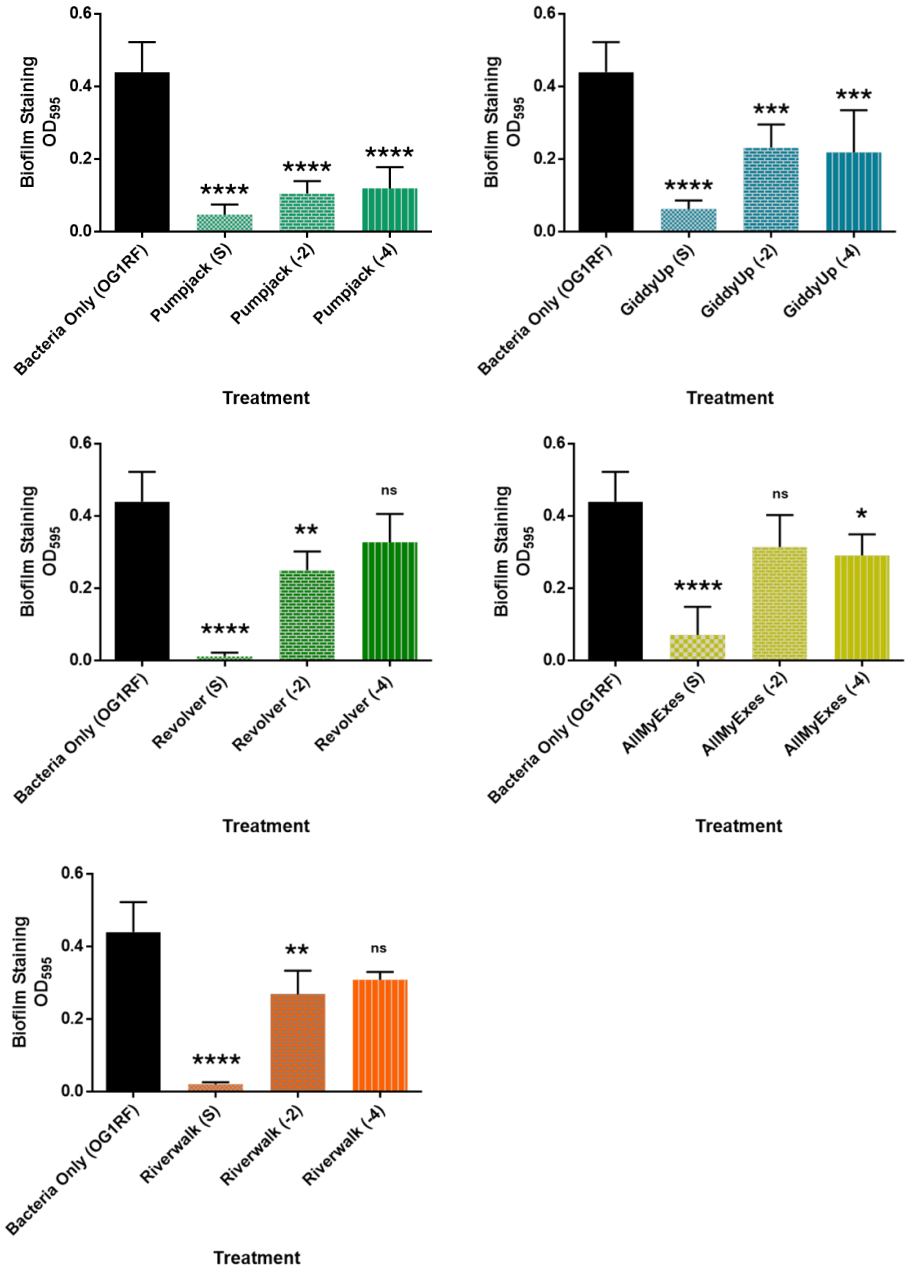


Figure 4. Disruption of OG1RF Produced Biofilm. Mature biofilms were grown over two weeks with an initial inoculation of 1.1×10^6 CFU of bacteria. Phage were added as (S) 10^7 PFU, (-2) 10^5 PFU, or (-4) 10^3 PFU and incubated for one week before crystal violet staining and measuring OD_{595}. An n= 3 was used for all phage treatments, (A) Pumpjack, (B) GiddyUp, (C) Revolver, (D) AllMyExes, and (E) Riverwalk. The bacteria only control had n = 7. Data were analyzed with a one-way ANOVA and Dunnett's post-hoc test (bacteria only comparison), ns = not significant, (*) $p \leq 0.05$, (**) $p \leq 0.01$, (***) $p \leq 0.001$, (****) $p \leq 0.00001$.

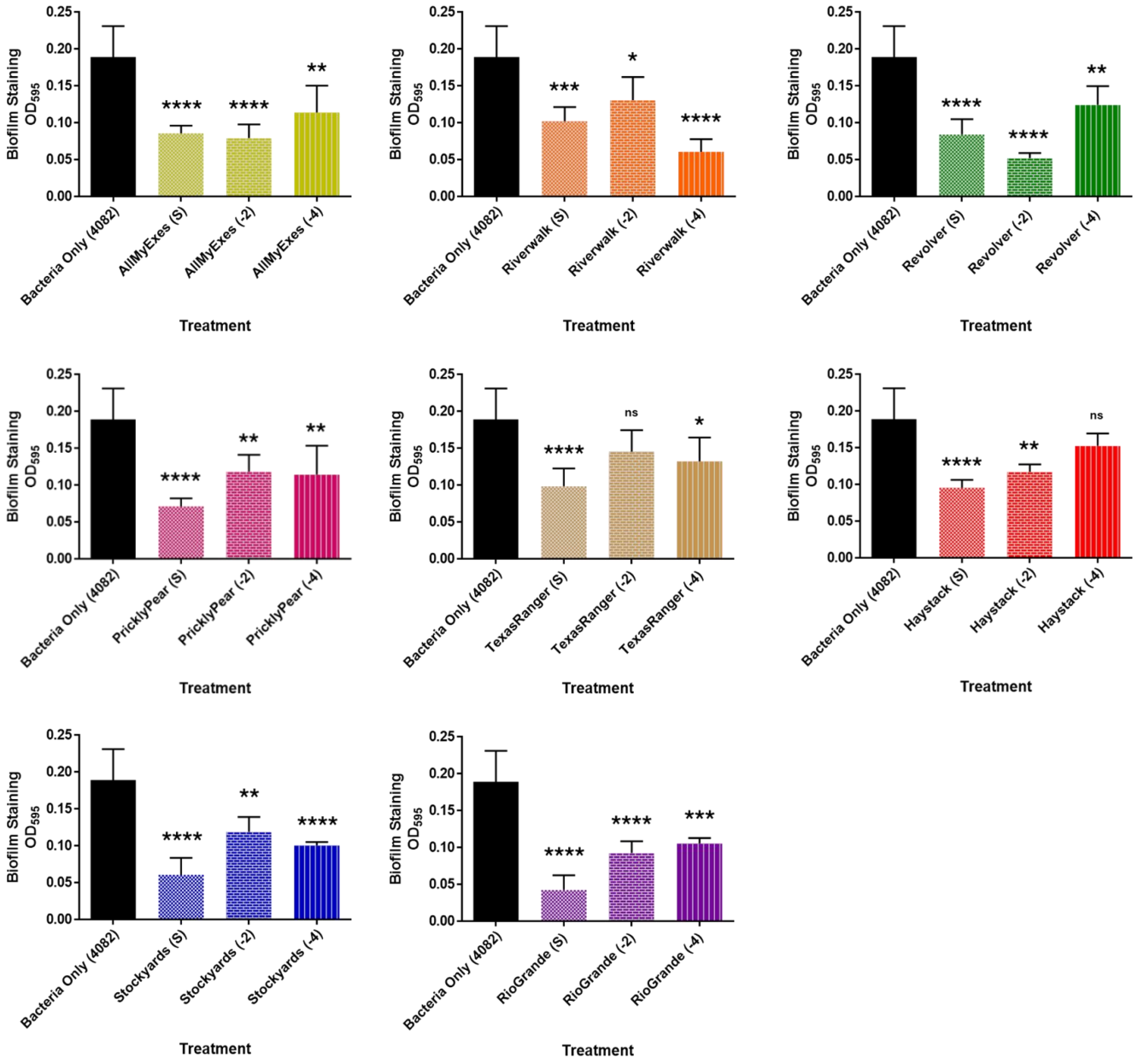
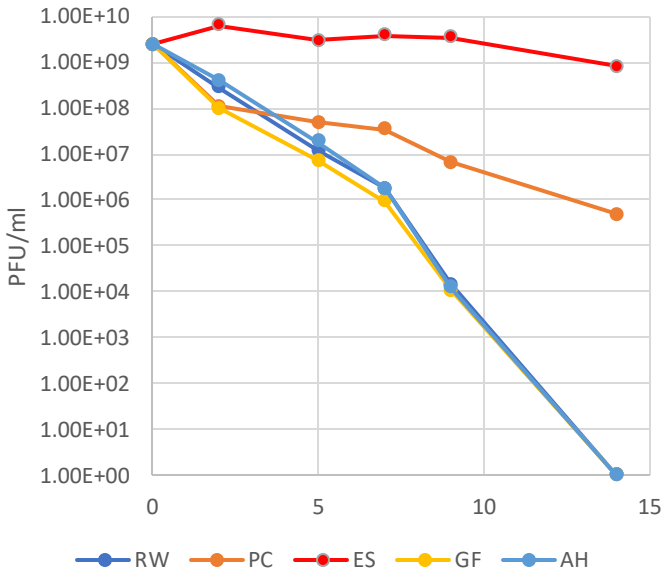
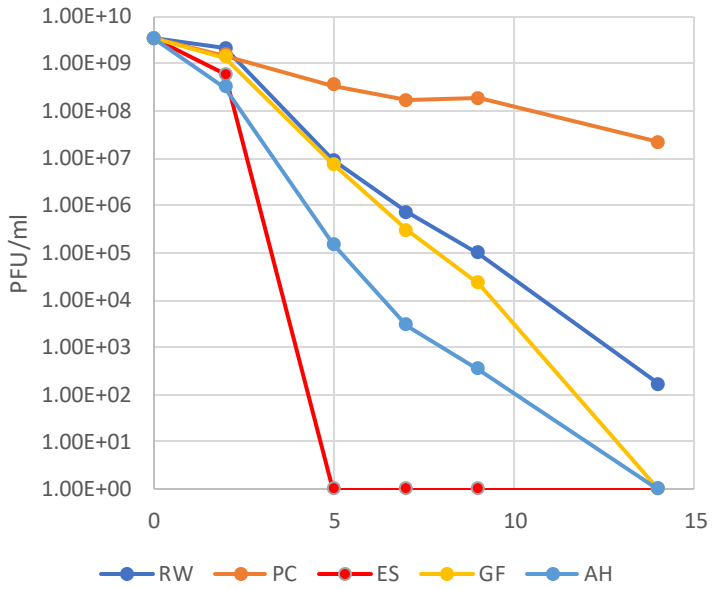


Figure 5. Disruption of 4082 Produced Biofilm. Mature biofilms were grown over two weeks with an initial inoculation of 1.1×10^6 CFU of bacteria. Phage were added as (S) 10^7 PFU, (-2) 10^5 PFU, or (-4) 10^3 PFU and incubated for one week before crystal violet staining and measuring OD_{595}. An n = 3 was used for all phage treatments, (A) AllMyExes, (B) Riverwalk, (C) Revolver, (D) PricklyPear, (E) TexasRanger, (F) Haystack, (G) Stockyards, (H) RioGrande. The bacteria only control had n = 12. Data were analyzed with a one-way ANOVA and Dunnett's post-hoc test (bacteria only comparison), ns = not significant, (*) $p \leq 0.05$, (**) $p \leq 0.01$, (***) $p \leq 0.001$, (****) $p \leq 0.00001$.



(A) Set Sealers and Phages



(B) Fresh Sealers and Phages

Figure 6. Stability of Riverwalk Phage Titer in Fresh and Set Endodontic Sealers. Assessment of phage viability over a 14-day incubation period. (A) Set Sealers: Phage Riverwalk remained highly stable in Pulp Canal Sealer and EndoSequence BC for the full 14 days, whereas titers in AH Plus Jet and GuttaFlow fell below detection limits (zero) after 9 days. (B) Fresh Sealers: Stability was highest when combined with Pulp Canal Sealer (14 days), followed by AH Plus Jet and GuttaFlow (up to 9 days), and EndoSequence BC (5 days).

PC: Pulp Canal, ES: Endosequence, GF: GuttaFlow, AH: AH Plus Jet

Conclusions

Integrating bacteriophages into root canal therapies is a highly viable strategy to eliminate persistent biofilm-forming bacteria.

- Highly Effective: Novel phages achieve up to 97% biofilm reduction in *E. faecalis.*
- Sealer-Compatible: Phages remain active and stable when combined with specific commercial root canal sealers (especially Pulp Canal Sealer).
- Operational Impact: Direct therapeutic integration could drastically reduce secondary root canal failures, preserving dental health on the frontlines.

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