HIV-1 protein coding sequences are present in relevant bacteria

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ABSTRACT

Human Immunodeficiency Virus (HIV) is a retrovirus that attacks the human immune system and can cause acquired immunodeficiency syndrome (AIDS). Early diagnosis and treatment of HIV infected individuals are considered key to reduce HIV transmission and developing AIDS. Subsequently, HIV diagnostics has become an important tool in the battle against AIDS. Most HIV tests are aimed to detect HIV antibodies and/or antigens, particularly p24, but there also tests that detect HIV genomic material. HIV tests are regarded as reliable and very specific. However, false-positives are known to occur, usually caused by infections with unrelated pathogens leading to cross-reactive antibodies. In this work, we found through TBLASTN searches that the genome of several bacterial species, most frequently *Klebsiella pneumoniae and Escherichia coli*, contain segments matching HIV-1 proteins, including p24 and other relevant proteins for HIV testing, with a high degree of similarity (up to 100 % sequence identity). The presence of HIV-1 in these bacteria of the human microbiota does not appear to an artifact, since HIV-1 proteins were detected in many isolates different strains/isolates of the same species. The proteome of other common viruses, particularly Influenza A virus and Hepatitis B virus, were also detected in bacterial genomes although to a much lesser extent. Overall, our findings support that some bacteria can acquire HIV-1 genetic material and could interfere with HIV-1 testing, causing false-positives.

Keywords: HIV, HIV diagnostics, Bacteria, *Klebsiella pneumoniae, Escherichia coli,* Cross-reactivity

34 **1. INTRODUCTION**

35

36 Human Immunodeficiency Virus (HIV) is an enveloped retrovirus that attacks $CD4^+$ T cells,
37 leading to their gradual depletion and the development of acquired immunodeficiency syndrome
38 (AIDS) (1,2). HIV transmission occurs mainly through sexual contact, often between men who
39 have sex with men but in Africa, where heterosexual sex transmission is dominant (3). The
40 virus can be categorized into 2 distinct subtypes: HIV type 1 (HIV-1) and HIV type 2 (HIV-2).
41 Both viruses are transmitted similarly, show the same tropism and cause AIDS (4). However,
42 infections by HIV-1 are much more common and progress quicker to AIDS than HIV-2
43 infections (4).

44

45 AIDS is the last stage of HIV infection, where the clinical symptoms of a weakened immune
46 system manifest (5,6). The first stage is the acute phase, which can pass unnoticed or manifest
47 with flu-like symptoms. During this phase, lasting 2-4 weeks after infection, the virus replicates
48 quickly, spreading through the body, and HIV genome and antigens, notably HIV-1 p24, can be
49 detected in blood. The second stage is the chronic or asymptomatic phase, in which the host
50 starts producing antibodies (Ab) and becomes HIV seropositive. In this stage, HIV antigens
51 drop and may be undetectable, yet viral replication and destruction of $CD4^+$ T cells continue and
52 patients eventually enter the AIDS stage unless they receive antiretroviral therapy (ART) (5,6).

53

54 There is evidence that starting ART early in the course of HIV infection has long-term health
55 benefits for patients and decreases the risk of transmission (7,8). Therefore, ART is currently
56 recommended for all HIV positive people, regardless of $CD4^+$ T cell count (9). Moreover, in
57 order to decrease the risk of infection, antiretroviral prophylaxis is also recommended for
58 perinatally-exposed newborns and people who has been exposed to contagious HIV-positive
59 material (9). Despite the noted benefits, ARTs do not eliminate the virus and have a
60 considerably degree of toxicity and long-term adverse effects (10). Thereby, patients in ART are
61 continuously monitored, and may require adjustments of the ART regime and additional

62 supportive treatments, all of which represent a considerable burden to both, patients and health
63 care systems (10).
64
65 Given the relevance of initiating HIV treatments early, HIV diagnostics have become an
66 important tool in the battle against HIV/AIDS. Moreover, these diagnostics are also useful to
67 stage and monitor patients, and for outbreak surveillance. HIV diagnostic has historically relied
68 on commercial enzyme immunoassays (EIAs) that detect antibodies to HIV antigens in serum
69 (5,11). Current generation of commercial HIV EIA tests (4-th and 5-th generation), detects Abs
70 against HIV-1 and HIV-2 as well as HIV-1 p24 antigen, which allows for earlier diagnosis of
71 HIV-1 infection (5). A list of FDA approved laboratory Antibody/Antigen (Ab/Ag) HIV tests
72 are available at https://www.cdc.gov/hiv/partners/testing/laboratorytests.html (accessed on
73 October/10/2024). In these tests, HIV-1 p24 is detected using Abs generated against this protein
74 or fragments derived from it, while HIV antibodies are detected using HIV antigens and/or
75 antigen fragment cocktails. The following antigens are generally considered for HIV-1 testing
76 (coding gene in italic): gp160 (envelope protein precursor, *env*), gp120 (extracellular region of
77 envelope protein), gp41 (transmembrane region of envelope protein), gp31 (immunodominant
78 region on integrase, *pol*), p51 (reverse transcriptase, *pol*), p24 (core protein, *gag*) and p17
79 (matrix protein, *gag*). In the laboratory, detection of Ab is sufficient to confirm HIV infection
80 but differentiation IEA tests may be necessary to distinguish between HIV-1 and HIV-2
81 infection. If the Ag test for HIV-1 p24 is positive but the Ab is negative, supplemental
82 confirmatory IEA tests (e.g. western-blotting), or RNA tests will be necessary to confirm or
83 negate HIV-1 infection (12). HIV RNA tests involve reverse transcription of HIV genetic
84 material into DNA and are aimed to detect conserved regions in *gag* or *pol* genes (5).
85
86 HIV tests are regarded as very reliable and specific. However, False Positive (FP) can occur
87 due to technical reasons (e.g. misinterpretation and clerical errors) and more importantly under
88 certain biological or medical conditions (13-15). Relevant conditions that have been reported to
89 interfere with HIV IEA tests and produce FP results include: pregnancy (16), recent vaccination

90 (e.g. flu, Tdap or Hepatitis B vaccine) (17-19), cancer (19), and autoimmune diseases like lupus
91 (20) or rheumatoid arthritis (20). Likewise, various infectious diseases (e.g. schistosomiasis,
92 malaria, COVID-19 and Epstein-Barr virus mononucleosis) have been shown to produce FPs
93 (21-24), likely due to cross-reactivity.
94
95 In this work, we investigated potential cross-reactivity between bacteria and HIV-1, using
96 TBLASTN searches against bacterial genomes with the HIV-1 proteome. Interestingly, we
97 detected the presence of HIV-1 proteome coding fragments in many bacterial species but more
98 frequently in *Klebsiella pneumoniae* and *Escherichia coli,* which are part of the human
99 microbiota. Sequence identity between HIV-1 and bacterial genomes was not only high (up to
100 100%) but reached the entire HIV-1 proteome, including p24 and other relevant proteins in
101 HIV-1 testing. Other common viruses were also detected in bacterial genomes but much more
102 sparsely. These results support that some bacteria can acquire HIV-1 sequences, which could
103 potentially induce immune cross-reactivity to HIV-1, causing FP results in HIV tests. Ruling out
104 this possibility will reduce the chance of putting healthy individuals on ART therapy and
105 alleviate the economic burden of HIV treatments on health care systems.
106

106 **2. METHODS**

107

108 **Genomes and proteins**

109

110 The HIV-1 reference genome (HIV-1 group M isotype B, isolate HXB2) was obtained from
111 NCBI (Accession: NC_001802), and amino acid coding sequences (CDS) and mature proteins
112 were collected upon it in a FASTA file. The entire proteomes of nine common human viruses,
113 consisting of the CDS in the relevant NCBI genomic sequences, were also collected in FASTA
114 files (Table 1).

115

116 **Table 1.** Proteomes of common human viruses considered in this study

Accession	Virus	CDS	Amino Acids
NC_038311	Human rhinovirus A	1	2157
NC_009996	Human rhinovirus C	1	2144
NC_038235	Respiratory Syncitial virus A	11	4536
NC_001781	Respiratory Syncitial virus B	11	4540
NC_026422_29*	Influenza A virus	12	4784
NC_002204_11*	Influenza B virus	10	4609
NC_003977	Hepatitis B virus	8	2299
M62321	Hepatitis C virus	1	3011
NC_027779	Papiloma virus	6	2309

117 * Proteome is contained in various independent accessions varying the last two digits and the range is
118 indicated. The corresponding author will provide the FASTA files upon written request.
119

120 **Microbial genome BLAST searches**

121

122 Sequence similarity searches of viral proteins in microbial genomes were carried online at
123 NCBI BLAST site (https://blast.ncbi.nlm.nih.gov/Blast.cgi), selecting TBLASTN. The amino
124 acid sequences of viral proteomes/proteins were entered as query and the RefSeq Genome
125 Database was selected as the target database, restricting the search to Bacteria (taxid:2).
126 TBLASTN searches were carried out with default settings but increasing the maximum target to

127 1000. The raw output from searches was downloaded as text and BLAST summary results with
128 taxonomic information were saved in Excel (Microsoft). The taxonomic TBLASTN information
129 tabulated the bacterial species with TBLASTN hits, indicating the number of hits and top bit-
130 score of all hits *per* bacteria as well.
131
132 Similar TBLASTN searches were carried out to determine bit-scores of viral proteomes/proteins
133 with themselves but restricting the search in RefSeq Genome Database to Viruses (taxid:10239).
134 As result, for each protein a self bit-score resulting from a hit alignment with itself was obtained
135 and recorded. Bit-scores only depend on the hit sequence alignment, not the size of the database.
136 Hence, the self bit-score (sBS) of a viral sequence query corresponds to the maximum bit-score
137 (mBS) that the query sequence can reach.
138
139 **Relative bit-scores**
140
141 Relative bit-scores (RBSs) of TBLASTN hits between viral protein queries and subject bacterial
142 genomic sequences were obtained by dividing bit-scores (BS) with the corresponding mBS, as
143 indicated in Equation 1.
144
145 $$RBS = \frac{BS}{mBS} \times 100 \qquad \text{Eq. 1}$$
146
147 Where mBS is the maximum bit-score of the relevant viral protein sequence obtained from a
148 self-hit alignment, as defined previously. Since a given viral protein query sequence can
149 produce numerous TBLASTN hits to bacterial sequences from the same species, the top RBS
150 for each viral protein per bacterial species was computed after the best hit, that with the top bit-
151 score.
152
153 **Viral proteome coverage**

154

155 The viral proteome coverage (VPC) was introduced as an estimation of the overall presence and

156 similarity of an entire viral proteome (*p*) in a given bacterial species (*b*). For any given viral

157 proteome used in TBLASTN searches, VPC (*p,b*) was obtained using Equation 2, which tallies

158 the top RBS (tRBS) from the best hit of each protein in the viral proteome (*p:1 .. N*) *per*

159 bacterial species (b), divided by the total number of proteins, N, in the viral proteome.

160

161 $$VPC_{(p,b)} = \frac{\sum_{p=N}^{p=1} tRBS_{(p,b)}}{N} \qquad \text{Eq.2}$$

162

163 **Other procedures**

164

165 TBLASTN results were parsed using PERL scripts to compute RBSs of hit alignments and data

166 was processed in Excel (Microsoft, Corp.). All graphics were generated using GraphPad Prism

167 (GraphPad Software, Inc.).

168

169 **3. RESULTS**

170

171 **Detection of HIV-1 proteins in bacterial genomes**

172

173 We investigated bacteria as potential sources of immune cross-reactivity with HIV-1 by
174 queering bacterial genomes with HIV-1 protein sequences, using TBLASTN (details in
175 Methods). The results revealed that all HIV-1 proteins produced highly significant TBLASTN
176 hits in the genome of various bacterial species (TBLASTN output description summary is
177 provided in Supplementary file 1). The single most significant hits with bacteria were produced
178 by p24, Vpr and Protease, which reached a relative bit-score (RBS) of 100% (Fig 1). The RBS
179 of a hit is computed by dividing the bit-score of the corresponding alignment between the viral
180 protein query and the subject genome by the maximum possible bit-score of the protein query,
181 obtained from a self-hit (details in Methods). Thus, an RBS of 100% for p24, Vpr and Protease
182 indicates that these proteins produce hit alignments spanning their entire length with 100%
183 sequence identity. As shown in Figure 1A, all HIV-1 proteins produced a considerable number
184 of hits with bacteria genomes with RBS ≥ 50 % and the RBS of the best hit was ≥ 60% for all
185 HIV-1 proteins. Interestingly, only a handful of bacterial species, including *Escherichia coli*
186 and *Klebsiella pneumoniae*, were the subject of the best hits with HIV-1 proteins (Fig.1). These
187 best hits between HIV-1 proteins and bacteria had a sequence identity ≥ 66.7 % and covered at
188 least 60% of the length of query (Vpu) (Table 1).

189

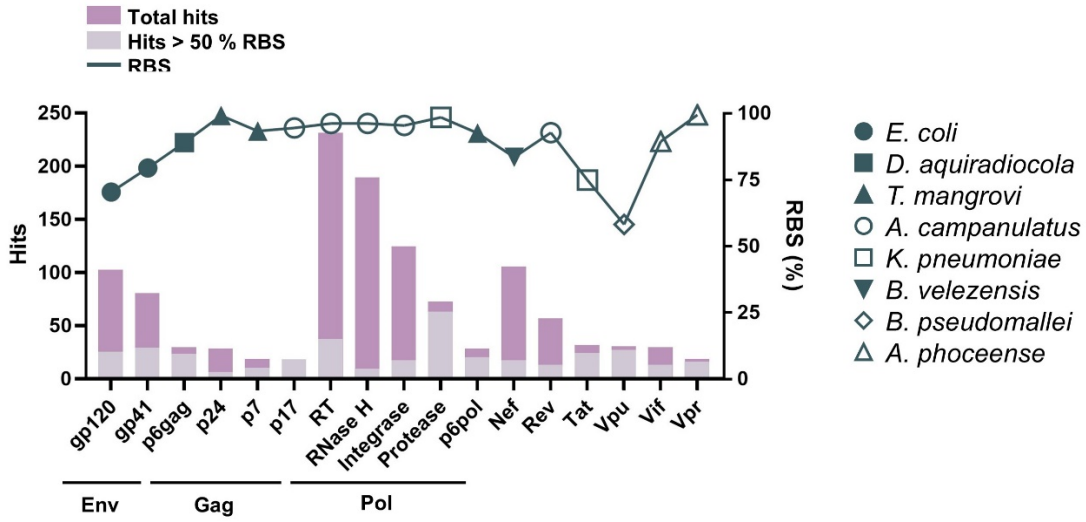

Figure 1. Presence of HIV-1 proteins in bacterial genomes. Chart showing for each HIV-1 protein (x-axis) the number of **TBLASTN** hits with bacterial genomes (left y-axis). Stacked columns in light grey show the number of hits with RBS ≥ 50%. The RBS (right y-axis) of the best hit for each HIV-1 protein is also plotted in the chart, using different symbols that represent the bacterial species, indicated on the right, subject of the hit. HIV-1 proteins that derive from Env, Gag and Pol are underlined.

Table 1. Statistics of best TBLASTN hits between HIV-proteins and bacterial genomes

Viral protein	Bacteria	E-value	Bit-Score	mBS[1]	RBS[2]	Q-coverage	Identity
gp120	*E. coli*	0	703	999	70.37%	99%	70.88%
gp41	*E. coli*	4×10−147	428	551	79.49%	92%	67.79%
p24	*T. mangrovi*	1×10−159	478	482	99.17%	100%	98.27%
p17	*A. campanulatus*	9×10−64	223	236	94.49%	100%	80.92%
p7	*T. mangrovi*	2×10−26	112	120	93.33%	100%	90.91%
p6gag	*D. aquiradiocola*	4×10−20	94.4	106	89.06%	100%	90.38 %
RT p51	*A. campanulatus*	0	1073	1096	97.9%	100%	94.29%
Protease	*K. pneumoniae*	3×10−56	199	202	98.51%	100%	97.98%
Integrase	*A. campanulatus*	0	591	602	98.17%	100%	97.22%
p15	*A. campanulatus*	5×10−67	231	240	96.25%	100%	95.83%
p6pol	*T. mangrovi*	9×10−20	92.8	99.8	92.98%	97%	95.74%
Nef	*B. velezensis*	9×10−108	325	389	83.54%	100%	73.79%
Rev	*A. campanulatus*	2×10−54	180	194	92.78%	100%	78.45%
Tat	*K. pneumoniae*	5×10−23	97.4	130	74.92%	83%	63.89%
Vif	*A. phoceense*	7×10−112	333	372	89.52%	98%	83.68%
Vpr	*A. phoceense*	2×10−58	193	193	100%	100%	96.88%
Vpu	*B. pseudomallei*	1×10−9	63.5	109	58.25%	65%	66.67%

[1] mBS is the maximum possible bit-score (BS) obtained from a self-hit alignment (details in Methods). [2] RBS, relative bit-score obtained dividing the BS by the corresponding mBS.

The genome database used in TBLASTN searches included not one but many genomes from the same bacterial species, usually different strains/isolates. Thereby, it is worth noting that HIV-1 proteins produced very similar hits in different representative genomes of a particular bacterial species (see Supplementary file 1). The number of hits per bacterial species with RBS ≥ 50% corresponding to HIV-1 proteins relevant for HIV-1 testing is shown in Figure 2 along with the RBS of the best hit. The selected proteins include gp120 and gp41, p24 and p17, p31 and p51.

201 The first two are part of the HIV-1 envelope glycoprotein (gp160 or Env) and derive by
202 cleavage, while p24 and p17 (matrix) derive from Gag protein, and p31 (integrase) and p51
203 (reverse transcriptase, RT) from Pol. The bacterial species whose genomes resulted in
204 significant hits varied with the distinct HIV-1 proteins (Fig.2). However, *E. coli* and *K.*
205 *pneumoniae* were the subject of the most significant hits with the majority of HIV-1 proteins.
206 Only p17 (matrix) did not yield any significant hit with these two bacterial species.

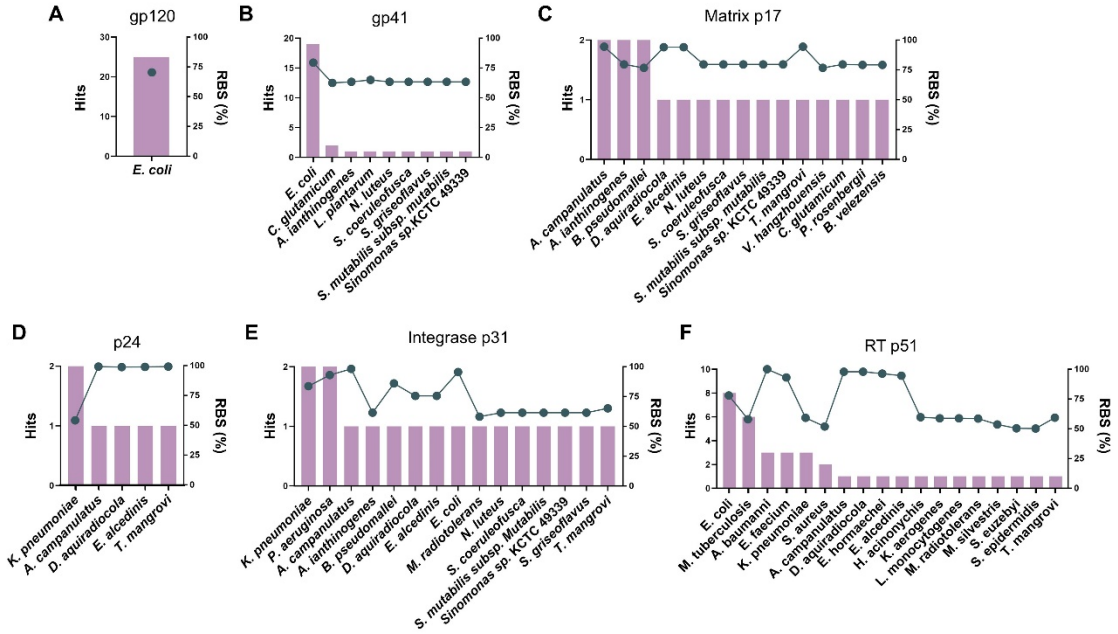

Figure 2. HIV-1 protein hits per bacterial species. The graphs show the number of hits on bacterial genomes (RBS ≥ 50%) of gp120 (**A**), gp41 (**B**), p17 (**C**), p24 (**D**), p51 (**E**), and p31 (**F**), stratified by bacterial species. The graphs also depict the RBS (line and dots) of the best hit per bacterial species.

207

208 **HIV-1 proteome coverage in bacterial genomes**

209 Since many HIV-1 proteins produced significant hits with the same bacterial species, we
210 introduced the viral proteome coverage (VPC) to estimate how much of the HIV-1 proteome,
211 not just a single protein, is present in the genome of a specific bacterial species. The VPC is
212 computed by adding up the RBS value from the best hit of each viral protein to the genome of a
213 specific bacterial species and dividing by the total number of viral proteins used in TBLASTN
214 searches (detailed in Methods). A VPC of 100% will indicate that all proteins included in the
215 viral proteome produce 100% identity hit alignments spanning their entire length. As shown in
216 Figure 3, HIV-1 reached the largest VPC in *K. pneumoniae* (67.74%), followed by *E. coli*

217 (63.72%), *Actinoplanes campanulatus* (60.12 %), *Enterococcus alcedinis* (59.91%) and
218 *Deinococcus aquiradiocola* (59.87%) However, while *A. campanulatus*, *E. alcedinis* and *D.*
219 *aquiradiocola* were the subject of only a few, yet highly significant hits*, K. pneumoniae and E.*
220 *coli* were the subject of the majority of significant hits, indicating that HIV-1 proteome appears
221 repeatedly in these two species and this presence is unlikely the result of contamination or
222 experimental artifacts. The data used to compute VPC values is provided in Supplementary
223 Table S1.

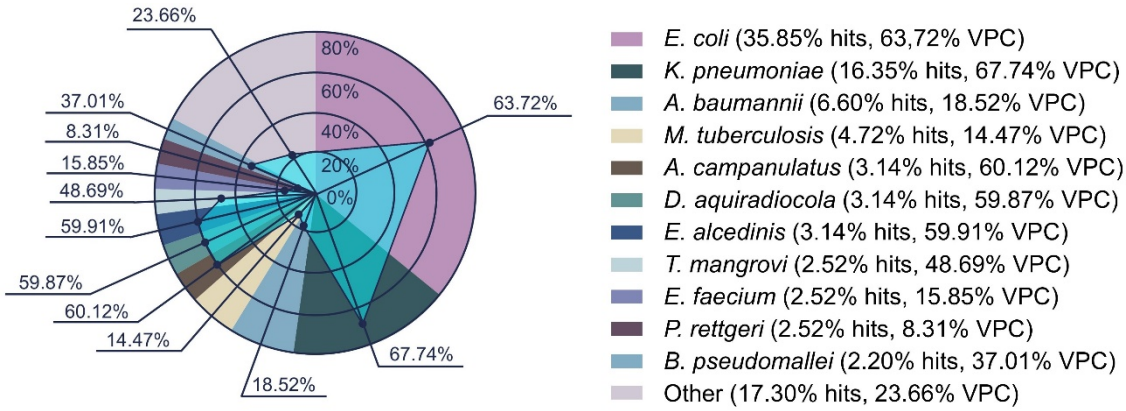

Figure 3. HIV-1 proteome in bacterial species. Parts-to-whole chart show the percentage of significant TBLASTN hits (RBS ≥ 50%) to each bacterial species considering all HIV-1 proteins. Total number of hits with ≥ 50% was 318. The spider charts represent the VPC per bacterial species computed after the best hits. The VPC shown in *Other* corresponds to the VPC determined on *Pseudomonas aeruginosa*, that reaches the largest VPC of all bacterial species included under *Other*.

224

225 **Proteome coverage of common viruses in bacterial genomes**

226

227 Given the extensive presence of HIV-1 in bacterial genomes, we examined whether the same
228 was true for other viruses. To that end, we carried out TBLASTN searches against bacterial
229 genomes with the proteome of common viruses and analyzed hits computing their RBS. The
230 VPC of the viruses per bacterial species was also computed after the RBS of the best hits. The
231 virus proteomes included Human Rhinovirus A (HRVA) and C (HRVC), Respiratory Syncitial
232 virus A (RSVA) and B (RSVB), Influenza A virus (IAV), Influenza B virus (IBV), Hepatitis B

233 virus (HBV), Hepatitis C virus (HCV) and Human Papiloma virus (HPV). The results revealed
234 that, all these viruses, with the exception of IAV, produced much fewer significant hits (RBS ≥
235 50%) in bacterial genomes than HIV-1 (Fig.4A). IAV produced a considerable number of
236 highly significant hits and reached a VPC of ≥ 75% in *Listeria monocytogenes* (Fig.4A). HBV
237 produced few but yet highly significant hits and actually reached a VPC of 100% in
238 *Streptomyces sp. 2BB-J2* (Fig.4). Supplementary Table S2 and S3 collect the statistics of the
239 best TBLASTN hits between IAV and HBV proteins with bacterial genomes, respectively.
240 Interestingly, we also noted that IAV produced a significant number of hits with *E. coli* reaching
241 a VPC ~60% (Fig.3B). Likewise, HBV reached a VPC of ~60% in *K. pneumoniae* (Fig.4C). In
242 supplementary Table S4 and S5, we provide the VPC data of IAV with *E. coli* and of HBV with
243 *K. pneumoniae*, respectively.

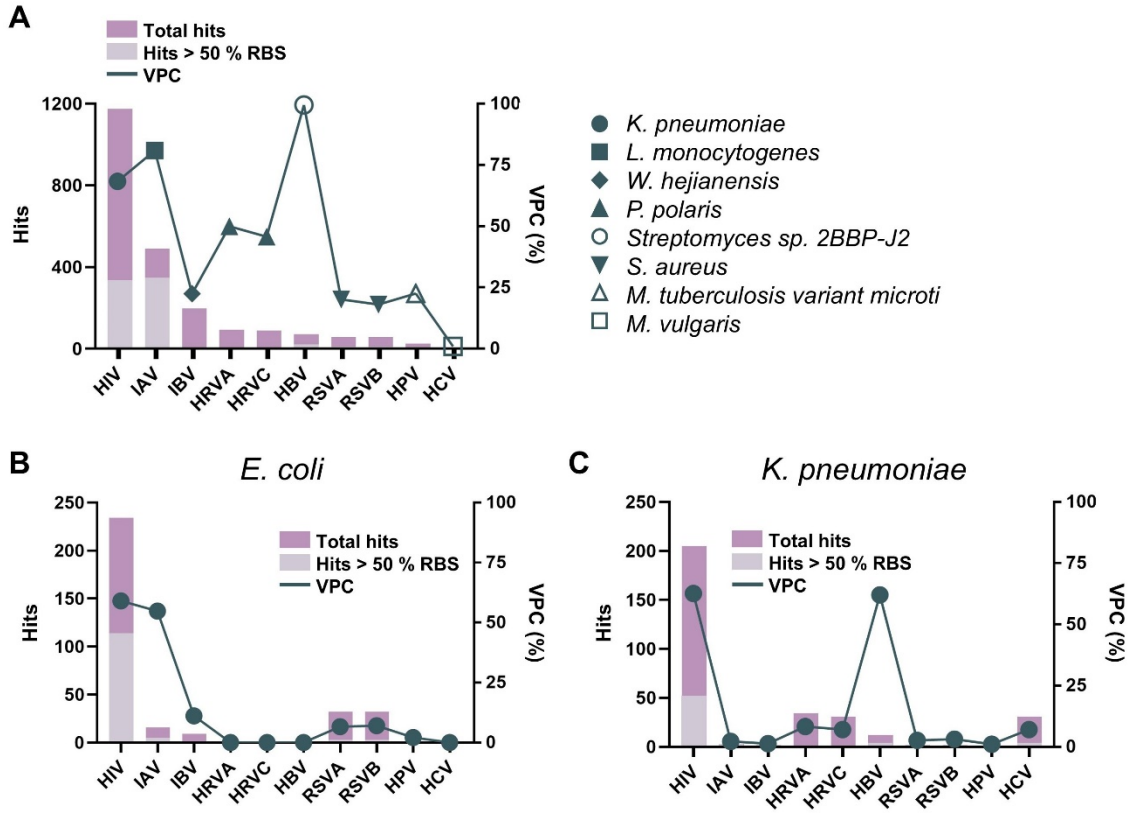

Fig.4. Presence of common virus proteomes in bacterial species. A) Chart showing for each virus (HIV-1, IAV IBV, HRVA, HRVC, RSVA, RSVB, HPV and HCV) (x-axis) the total number of TBLASTN hits with bacterial genomes (left y-axis). Stacked columns in light grey show the number of hits with RBS ≥ 50%. The largest VPC value (right y-axis) reached by the viruses is also plotted in the chart (right y-axis), using different symbols that represent the bacterial species (shown on the right) where that particular value was attained. **B**) and **C**) show the same data as in panel A but limited to TBLASTN hits with *E. coli* and *K. pneumoniae*,

respectively.

244

244 **4. DISCUSSION**

245

246 HIV testing is an important part in the fight against AIDS since early diagnosis and treatment
247 maximize the benefits of ART therapy (7,8). Thereby, major attention is paid to minimize false-
248 negatives in HIV tests results that could delay ART therapy. However, FPs in HIV testing are
249 not without consequences, including those derived from exposing healthy individuals to the
250 adverse effects of lifelong treatments, not to mention the significant social and emotional impact
251 on a person. Fortunately, FPs are considered rare, yet they are known to occur. Therefore
252 important to be aware of the circumstances under which they may arise.

253

254 FP HIV results are more commonly reported under some infections that induce antibodies cross-
255 reacting with HIV antigens (15). These cross-reactive antibodies likely result from antigens
256 showing molecular mimicry with HIV-1 proteins, which can be readily detected by sequence
257 similarity (25-27). However, the infectious agents that can potentially induce cross-reactive
258 antibodies with HIV remain to be investigated and are unknown. In this work, we investigated
259 bacteria as potential sources of immune cross-reactivity with HIV-1 due to molecular mimicry,
260 using TBLASTN searches with HIV-1 proteins against bacterial genomes. The results were
261 surprising and unexpected.

262

263 We found that HIV-1 proteins appear to be present in quite a few bacterial species (Fig.1, Fig.2
264 and Fig.3). Proteins like p24, Vpr and Integrase were found to produce 100% sequence identity
265 hit alignments over their entire length of the sequences (relative bit-score, RBS, of 100 %), and
266 hit alignments produced by other HIV-1 proteins were also highly significant (RBS ≥ 60 %)
267 (Fig. 1). *K. pneumoniae* and *E. coli* were the subject of the best hits with HIV-1 proteins that
268 are relevant for HIV testing like gp120, gp41, p24, p31 and p51 (Fig. 2). *K. pneumoniae* and *E.*
269 *coli* also produced highly significant hits with the remaining HIV-1 proteins, reaching a VPC of
270 67.74% and 63.72%, respectively. Only three other bacterial species, *A. campanulatus, E.*

271 *alcedinis and D. aquiradiocola*, reached a comparable VPC of 60.12 %, 59.91%and 59.87%,
272 respectively.
273
274 Arguably, the detection of bacterial genomic sequences that are highly similar (if not identical)
275 to HIV-1 proteins or derived fragments could be attributed to contamination or some other
276 experimental artifact. However, the fact that the same/similar highly significant HIV-1 hits
277 were detected in different strains/isolates of the same bacterial species, such as in the case of *K.*
278 *pneumoniae* and *E. coli,* makes this possibility unlikely. Furthermore, the presence of HIV-1
279 related sequences in bacterial genomes might be greater if sequences that are not aligned with
280 the relevant taxa are excluded during annotation and curation processes. In comparison with
281 HIV-1, the proteome of other common viruses was seldom detected in bacterial genomes with
282 the exception of Influenza virus A (IAV) and Hepatitis B virus (HBV) (Fig. 4). Interestingly, *K.*
283 *pneumoniae* and *E. coli* also capitalized a relevant number of highly significant hits with IAV
284 and HBV but with differences. Thus, IAV reached a VPC of ~ 60% in *E. coli* and of 20% *K.*
285 *pneumoniae.* In contrast, HPV reached a VPC of ~60% in *K. pneumoniae* while in *E. coli* was ≤
286 20%. Overall, these data support that bacteria including *K. pneumoniae and E. coli* can acquire
287 viral genomic sequences with perhaps certain preferences. This possibility has actually been
288 verified experimentally for HIV-1. Thus, Zajac *et al.* (28) reported that DNA isolated from
289 intestinal bacteria of HIV/AIDS patients bears sequences that are more than 90% identical with
290 those of HIV-1. Of note both *E. coli* and *K. pneumoniae* are part of the human gut microbiota,
291 where they are usually harmless (29,30). However, there are strains of *E. coli* that are
292 pathogenic (31) and *K. pneumoniae* can colonize other tissues and produce an ample range of
293 diseases (30). In general, bacteria in the gut microbiota can cause harm if they enter the
294 organism and are repelled by the immune system. Indeed, antibodies against the gut microbiota
295 are very common in human sera (32), which surely result after occasional breaches in the gut
296 mucosa leading to entrance and immune recognition.
297

298 Viruses, particularly retroviruses, are well known vectors of horizontal gene transfer, posing an
299 important evolutionary force in most life forms, including bacteria (33,34). However, how
300 bacteria can integrate sequences from viruses that infect humans is unclear –more so in the case
301 of RNA viruses– and deserves to be investigated. Nonetheless, human commensal bacteria,
302 including *E. coli,* are known to interact with enteric viruses (35) and likely with other viruses.
303 Hence, we can speculate that such close interactions at the gut, or sites of infection, may also
304 facilitate gene transfer from viruses to bacteria. Alternatively, viral gene transfer may occur
305 between bacteria and virus-infected cells (33). Integration of genomic material from RNA
306 viruses into bacterial genome will require prior reverse transcription to DNA. Incidentally, *E.*
307 *coli* has been found to express a functional reverse transcriptase (36) and likewise *K.*
308 *pneumoniae* (37), which is part of a viral defense mechanism (38).
309
310 Integration of HIV-1 sequences in *K. pneumoniae* and *E. coli* can have wide implications, since
311 those bacteria are part the gut microbiota and can be transmitted through person-to-person
312 contact or contact with contaminated sources. Bacteria carrying HIV-1 sequences are very
313 unlikely to shed infectious viral particles and contribute to HIV-1 transmission. However, *K.*
314 *pneumoniae* and *E. coli* specimens bearing HIV-1 sequences could serve as transmission vectors
315 of HIV positivity, provided that the bacteria can express HIV-1 proteins. Interestingly, this
316 appears to be the case. By using HIV-1 antibodies against various Gag derived proteins,
317 Hainova *et al.* (39) detected a protein in bacteria isolated from HIV-1 patients with size
318 comparable to Gag precursor. It could be argued that bacteria expressing HIV-1 proteins will
319 unlikely secrete them, which may be anticipated as necessary to induce HIV-1 antibodies and
320 transmit HIV positivity. However, once bacteria enter the body there are processes that destroy
321 them and result in the release of hidden antigens and epitopes, which can then induce specific
322 antibodies. Indeed, a recent work has revealed that the majority of antibodies elicited during
323 infection are directed against hidden antigens and epitopes (40). Relevant immune processes
324 that would lead to particulate/degrade bacteria and antigens include complement activation (41)
325 and phagocytosis (42).

326 **5. CONCLUSION**

327

328 Our work supports that highly common and clinically relevant bacteria such as *E. coli* and *K.*
329 *pneumoniae* can acquire HIV-1 sequences and may transmit HIV positivity to people. Since
330 these bacteria are present in the gut microbiota, transmission of HIV positivity without actual
331 HIV infection may be common: it could occur through person-to-person contacts, from
332 contaminated sources and procedures such as microbiota transplantation, which demands testing
333 the presence of HIV sequences in microbiota samples. The possibility that bacteria such as *E.*
334 *coli* and *K. pneumoniae* could transmit HIV positivity deserves serious consideration to prevent
335 treating healthy individuals with life-long medications that carry significant toxicity, and take a
336 heavy toll on health care systems. Therefore, it is crucial to count with accurate HIV
337 diagnostics guaranteeing that life-saving ART therapy is only received by HIV infected patients.

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475 **SUPPLEMENTARY MATERIALS**

476

477 **Supplementary File 1**: TBLASTN description summary of HIV-1 proteins against bacterial

478 genomes.

479

480 **Supplementary File 2:** Supplementary Tables S1, S2, S3, S4 and S5.

481 Supplementary Table S1: HIV-1 proteome coverage in *E. coli* and *K. pneumoniae*.

482 Supplementary Table S2. IAV proteome coverage in *L. monocytogenes*.

483 Supplementary Table S3. HBV proteome coverage in *Streptomyces sp. 2BBP J2*.

484 Supplementary Table S4. IAV proteome coverage in *E. coli*.

485 Supplementary Table S5. HBV proteome coverage in *K. pneumoniae*

486

487 Author Contributions: Conceptualization: P.A.R; Methodology: H.F.P.-P. & J.M.-G.;
488 Investigation: P.A.R & E.M.L; Data Analysis: H.F.P.-P. & J.M.-G; Writing-Original Draft:
489 P.A.R; Final Writing & Editing: H.F.P.-P., J.M.-G, E.M.L & P.A.R. All authors have read and
490 approved the final manuscript.

491 Funding: This research project was performed with no funding.

492 Institutional Review Board Statement: Not applicable.

493 Informed Consent Statement: Not applicable.

494 Competing interest: The authors declare no competing interest

495 Author Approvals: All the individuals named as authors of this manuscript have approved its

496 submission to bioRxiv