

# Diversity of Sequence Types and Impact of Fitness Cost among Carbapenem-Resistant *Acinetobacter baumannii* Isolates from Tripoli, Libya

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**ABSTRACT** We investigated the molecular epidemiology of 21 carbapenem-resistant *Acinetobacter baumannii* isolates from Libya and assessed their relative fitness. Core genome multilocus sequence typing (MLST) revealed five interhospital transmission clusters. Three clusters were associated with the international clones (IC) IC1, IC2, and IC7. Carbapenem-resistance was associated with *bla*<sub>OXA-23</sub>, *bla*<sub>GES-11</sub>, or *bla*<sub>NDM-1</sub>. Compared to that of *A. baumannii* DSM 30008, the doubling time was similar over 10 h, but after 16 h, half the isolates grew to higher densities, suggesting a fitness advantage.

**KEYWORDS** *Acinetobacter baumannii*, antimicrobial resistance, carbapenems, fitness cost

*Acinetobacter baumannii* causes life-threatening hospital-acquired infections among immunocompromised and critically ill patients (1). In addition to being a common nosocomial pathogen, rising rates of *A. baumannii* infections correlate with armed conflicts, especially during periods of heightened combat intensity (2–6). Libya has experienced a protracted civil war since 2011. The aim of this study was to investigate the molecular epidemiology of *A. baumannii* isolates from patients hospitalized in Tripoli, Libya, to identify their carbapenem resistance mechanisms and to assess the fitness cost to these isolates.

A total of 23 *A. baumannii* isolates, confirmed using the *gyrB* multiplex PCR (7), were collected from patients at three hospitals in Tripoli, Libya (Table 1). Table 2 summarizes antimicrobial susceptibility testing results by broth microdilution according to CLSI guidelines (8). One isolate was resistant to colistin, 12 were resistant to amikacin and tetracycline, 16 to trimethoprim-sulfamethoxazole, 21 to meropenem and imipenem (91%), and 22 were resistant to levofloxacin. All were resistant to ceftazidime, cefepime, gentamicin, ciprofloxacin, and piperacillin-tazobactam. In a previous study from Libya, 107 isolates of 167 (64%) were carbapenem-resistant *A. baumannii* (CRAB) (9).

Whole-genome sequencing and core genome multilocus sequence typing (cgMLST) were performed as previously described (10). Pasteur sequence types were determined using the pubMLST website (<https://pubmlst.org/abaumannii/>). Resistomes were identified using ResFinder. The ADC and OXA variants were identified using the BLAST function

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**TABLE 1** Summary of the 23 *A. baumannii* isolates and sources

Isolate	Hospital <sup>a</sup>	Gender <sup>b</sup>	Age	Patient <sup>c</sup>	Ward <sup>d</sup>	Sample <sup>e</sup>	TC <sup>f</sup>	ST <sup>g</sup>
N22	BPSH	M	43 yr	In	ICU	Wound	A	ST25
N34	TMC	F	60 yr	In	RHM	Urine		
N46	BPSH	M	32 yr	In	MICU	W/S		
N98	TMC	M	70 yr	In	GSICU	Wound		
N33	BPSH	M	37 yr	Out	ICU	W/S		
N3	BPSH	M	35 yr	In	ICU	Wound	B	ST136
N60	BPSH	M	32 yr	In	ICUB	W/S		
N103	BPSH	M	25 yr	In	ICUB	W/S		
N19	BPSH	M	32 yr	In	ICU	Wound	C	ST1
N20	BPSH	M	27 yr	In	ICU	Wound		
N44	TMC	F	40 yr	In	GSICU	FC tip		
N13	BPSH	M	35 yr	In	ICU	Wound	Singleton	
N51	TMC	M	12 yr	In	PO	Blood	Singleton	
N54	BPSH	M	23 yr	In	ICUB	Blood	E	ST2
N57	TMC	M	61 yr	In	GSICU	Sputum		
N99	TMC	F	Newborn	In	SCBU	Vaginal swab		
N27	BPSH	M	25 yr	In	ICU	Wound	Singleton	
N52	BPSH	M	35 yr	In	ICUB	Blood	Singleton	
N25	BPSH	M	37 yr	In	ICU	Wound	Singleton	

<sup>a</sup>BPSH, burn and pediatric surgery hospital; TMC, Tripoli Medical Center; CHT, Central Hospital of Tripoli.

<sup>b</sup>M, male; F, female.

<sup>c</sup>IN, inpatient; OUT, outpatient.

<sup>d</sup>ICU, intensive care unit; RHM, rheumatology; MICU, medical ICU; GSICU, general surgery ICU; ICUB, ICU unit B; SCBU, special care baby unit; PO, Prenatal oncology.

<sup>e</sup>W/S, wound swab; Throat/s, throat swab; FC tip, Foley catheter tip.

<sup>f</sup>TC, transmission cluster.

<sup>g</sup>ST, sequence type.

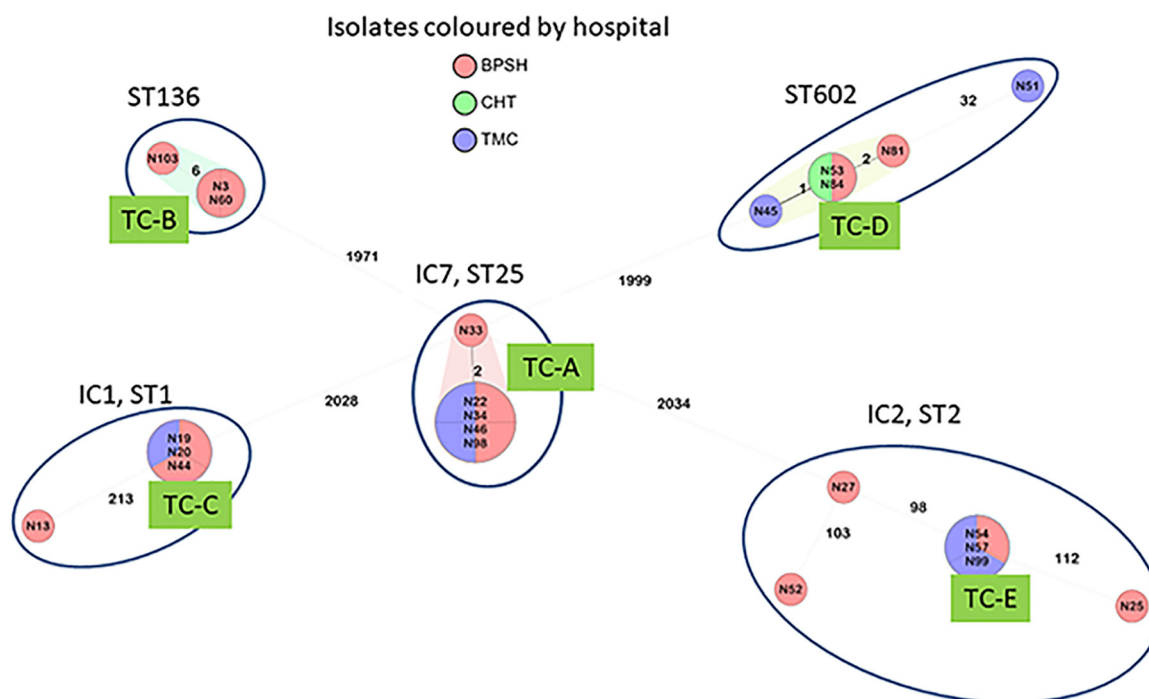
of the beta-lactamase database (<http://www.bldb.eu/>). The novel insertion (IS) element IS*Aba58* was identified using ISfinder (<https://www-is.biotoul.fr/>). Mutations in *gyrA* and *parC* associated with fluoroquinolone resistance were detected manually.

The isolates were assigned to five sequence types (STs) (Table 1), three of which are associated with international clones (IC): ST1 (IC1), ST2 (IC2), and ST25 (IC7). cgMLST results are summarized in Fig. 1. Transmission clusters (TCs) suggest interhospital transmissions, with TC-D involving all three hospitals. The five IC7 isolates formed a TC from five different wards at two of the three hospitals, suggesting patient movement or an endemic clone circulating between the hospitals. Interhospital TCs were also seen within the IC1, IC2, and the ST136 groups. However, not all isolates within a ST were part of a TC. Several studies have highlighted a wide diversity of STs associated with CRAB in Libya (11–13). Notably, ST25 was missing from previous studies, possibly reflecting a recent importation into Libya.

Table 3 summarizes the antimicrobial resistance determinants. Generally, isolates that clustered together had similar resistomes, with a few exceptions. The four identical

**TABLE 2** Antimicrobial susceptibility testing results of all the 23 *A. baumannii* isolates

Antibiotic	No. of isolates resistant (%)	MIC (µg/ml)		
		Range	50%	90%
Meropenem	21 (91)	<1 to 128	32	64
Imipenem	21 (91)	2 to 128	64	128
Ceftazidime	23 (100)	32 to >1,024	>1,024	>1,024
Cefepime	23 (100)	32-1,024	256	512
Gentamicin	23 (100)	8 to >1,024	256	>1,024
Ciprofloxacin	23 (100)	8 to 256	32	64
Levofloxacin	22 (96)	2 to 64	8	16
Colistin	1 (4)	<1 to 8	<1	<1
Amikacin	12 (52)	2 to >1,024	32	64
Piperacillin-tazobactam	23 (100)	128 to 1,024	512	1,024
Trimethoprim-sulfamethoxazole	16 (70)	0.5 to >64	4	>64
Tetracycline	12 (52)	<1 to 1,024	16	1,024



**FIG 1** cgMLST of the 23 *A. baumannii* isolates.

isolates in TC-A differ from isolate N33 by 2 alleles, and N33 is also missing the sulfonamide resistance gene *sul2*. Isolate N45 (TC-D), while only one allele distant from N53 and N84, was carbapenem susceptible and was missing *bla*<sub>OXA-23</sub> as well as aminoglycoside and macrolide resistance determinants.

All isolates possessed the intrinsic cephalosporinase *bla*<sub>ADC</sub> (not shown). There was a good correlation between the STs/IC and the OXA-51-variant: IC1 (*bla*<sub>OXA-69</sub>), IC2 (*bla*<sub>OXA-66</sub>), IC7 (*bla*<sub>OXA-64</sub>), and ST136 (*bla*<sub>OXA-378</sub>). All of the ST602 isolates had the novel *ISAb58* inserted in their intrinsic *bla*<sub>OXA-51-like</sub>. All CRAB in this study carried an acquired carbapenemase. The most common was *bla*<sub>OXA-23</sub> ( $n = 18$ ), six isolates in addition also possessed *bla*<sub>GES-11</sub>. Three isolates possessed *bla*<sub>NDM-1</sub> as their sole carbapenemase. The two carbapenem-susceptible isolates had only their intrinsic *bla*<sub>OXA</sub>. *ISAb1* was not associated with the intrinsic *bla*<sub>OXA</sub> in any isolate. In two previous studies assessing CRAB in Libya, *bla*<sub>OXA-23</sub> was the predominant carbapenemase in both, with the second most common being *bla*<sub>OXA-24</sub> (11) or *bla*<sub>NDM-1</sub> (12), the latter being similar to our findings.

All isolates had a large array of aminoglycoside resistance genes. Isolates with tetracycline MICs of  $\geq 256 \mu\text{g/ml}$  ( $n = 11$ ) carried *tetB* or *tetA*. One isolate with a tetracycline MIC of  $16 \mu\text{g/ml}$  (N27) had no known tetracycline resistance determinant. Ciprofloxacin MICs of  $\geq 16 \mu\text{g/ml}$  were associated with a Ser83-Leu substitution in GyrA and Ser80-Leu in ParC, while isolate N27 with a ciprofloxacin MIC of  $256 \mu\text{g/ml}$  had an additional substitution in GyrA, Glu87-Val. One isolate with a ciprofloxacin MIC of  $8 \mu\text{g/ml}$  had the GyrA substitution, but in ParC, Ser80-Ala. The so-called fluoroquinolone resistance gene *aac(6')Ib-cr* was found in some isolates but was not associated with higher ciprofloxacin or levofloxacin MICs.

To determine the relative fitness of the isolates, fresh cultures were incubated overnight on MacConkey agar at  $37^\circ\text{C}$  and used the following day to inoculate 10 ml of sterile cation-adjusted Mueller-Hinton broth. After incubation overnight at  $37^\circ\text{C}$ , each culture was diluted 1:1,000 in fresh broth, and 200- $\mu\text{l}$  aliquots were transferred into 4 separate wells of a 96-well microtiter plate. The growth rate was measured for 16 h with reads at 30-min intervals using a densitometer (optical density at 600 nm [OD<sub>600</sub>]).

**TABLE 3** Antibiotic resistance determinants and GyrA/ParC substitutions<sup>a</sup>

Isolate	Beta-lactam gene(s)	Aminoglycoside gene(s)	Sulphonamide gene(s)	FQ/AMG gene <sup>b</sup>	Phenicol gene	TET gene <sup>c</sup>	TMP gene <sup>d</sup>	Macrolide gene(s)	GyrA substitution	ParC substitution
N22	<i>bla</i> <sub>OXA-23*</sub> , <i>bla</i> <sub>OXA-64</sub>	<i>aadB</i> -like, <i>aph(3'')-Ic</i> , <i>aph(3'')-Vla</i> -like, <i>strA</i> , <i>strB</i>	<i>sul2</i>						S83-L	S80-L
N34	<i>bla</i> <sub>OXA-23*</sub> , <i>bla</i> <sub>OXA-64</sub>	<i>aadB</i> -like, <i>aph(3'')-Ic</i> , <i>aph(3'')-Vla</i> -like, <i>strA</i> , <i>strB</i>	<i>sul2</i>						S83-L	S80-L
N46	<i>bla</i> <sub>OXA-23*</sub> , <i>bla</i> <sub>OXA-64</sub>	<i>aadB</i> -like, <i>aph(3'')-Ic</i> , <i>aph(3'')-Vla</i> -like, <i>strA</i> , <i>strB</i>	<i>sul2</i>						S83-L	S80-L
N98	<i>bla</i> <sub>OXA-23*</sub> , <i>bla</i> <sub>OXA-64</sub>	<i>aadB</i> -like, <i>aph(3'')-Ic</i> , <i>aph(3'')-Vla</i> -like, <i>strA</i> , <i>strB</i>	<i>sul2</i>						S83-L	S80-L
N33	<i>bla</i> <sub>OXA-23*</sub> , <i>bla</i> <sub>OXA-64</sub>	<i>aadB</i> -like, <i>aph(3'')-Ic</i> , <i>aph(3'')-Vla</i> -like, <i>strA</i> -like, <i>strB</i>							S83-L	S80-L
N3	<i>bla</i> <sub>GES-11*</sub> , <i>bla</i> <sub>OXA-23*</sub> , <i>bla</i> <sub>OXA-378</sub>	<i>aadA2</i> , <i>aadB</i> , <i>aph(3'')-Vla</i> -like, <i>strA</i> -like, <i>strB</i> -like, <i>aacA4</i> -like	<i>sul1</i>	<i>aac(6'')/lb-cr</i> -like	<i>cmIA1</i> -like		<i>dfra7</i>		S83-L	S80-L
N60	<i>bla</i> <sub>GES-11*</sub> , <i>bla</i> <sub>OXA-23*</sub> , <i>bla</i> <sub>OXA-378</sub>	<i>aadA2</i> , <i>aadB</i> , <i>aph(3'')-Vla</i> -like, <i>strA</i> -like, <i>strB</i> -like, <i>aacA4</i> -like	<i>sul1</i>	<i>aac(6'')/lb-cr</i> -like	<i>cmIA1</i> -like		<i>dfra7</i>		S83-L	S80-L
N103	<i>bla</i> <sub>GES-11*</sub> , <i>bla</i> <sub>OXA-23*</sub> , <i>bla</i> <sub>OXA-378</sub>	<i>aadA2</i> , <i>aadB</i> , <i>aph(3'')-Vla</i> -like, <i>strA</i> -like, <i>strB</i> -like, <i>aacA4</i> -like	<i>sul1</i>	<i>aac(6'')/lb-cr</i> -like	<i>cmIA1</i> -like		<i>dfra7</i>		S83-L	S80-L
N19	<i>bla</i> <sub>GES-11*</sub> , <i>bla</i> <sub>OXA-23*</sub> , <i>bla</i> <sub>OXA-69</sub>	<i>aadA2</i> , <i>aadB</i> , <i>aph(3'')-Vla</i> -like, <i>strA</i> -like, <i>strB</i> -like, <i>aacA4</i> -like	<i>sul1</i>	<i>aac(6'')/lb-cr</i> -like	<i>cmIA1</i> -like		<i>dfra7</i>		S83-L	S80-L
N20	<i>bla</i> <sub>GES-11*</sub> , <i>bla</i> <sub>OXA-23*</sub> , <i>bla</i> <sub>OXA-69</sub>	<i>aadA2</i> , <i>aadB</i> , <i>aph(3'')-Vla</i> -like, <i>strA</i> -like, <i>strB</i> -like, <i>aacA4</i> -like	<i>sul1</i>	<i>aac(6'')/lb-cr</i> -like	<i>cmIA1</i> -like		<i>dfra7</i>		S83-L	S80-L
N44	<i>bla</i> <sub>GES-11*</sub> , <i>bla</i> <sub>OXA-23*</sub> , <i>bla</i> <sub>OXA-69</sub>	<i>aadA2</i> , <i>aadB</i> , <i>aph(3'')-Vla</i> -like, <i>strA</i> -like, <i>strB</i> -like, <i>aacA4</i> -like	<i>sul1</i>	<i>aac(6'')/lb-cr</i> -like	<i>cmIA1</i> -like		<i>dfra7</i>		S83-L	S80-L
N13	<i>bla</i> <sub>CARB-8</sub> , <i>bla</i> <sub>OXA-23</sub> , <i>bla</i> <sub>OXA-69</sub> , <i>bla</i> <sub>TEM-1D</sub>	<i>aac(3'')-Ia</i> -like, <i>aadA1</i> , <i>aph(3'')-Ic</i> , <i>aph(3'')-Vla</i> -like	<i>sul1</i>		<i>catA1</i> -like	<i>tet(A)</i>			S83-L	S80-A
N45	<i>bla</i> <sub>OXA-317</sub> , <i>bla</i> <sub>PER-1</sub>	<i>aadB</i> -like, <i>strA</i> -like, <i>strB</i>	<i>sul2</i>			<i>tet(B)</i>			S83-L	S80-L
N53	<i>bla</i> <sub>OXA-317</sub> , <i>bla</i> <sub>PER-1</sub> , <i>bla</i> <sub>OXA-23</sub>	<i>aadB</i> -like, <i>aph(3'')-Vla</i> -like, <i>aph(3'')-Vlb</i> , <i>strA</i> -like, <i>strB</i> -like	<i>sul2</i>			<i>tet(B)</i>		<i>mph(E)</i> , <i>msr(E)</i>	S83-L	S80-L
N81	<i>bla</i> <sub>OXA-317</sub> , <i>bla</i> <sub>PER-1</sub> , <i>bla</i> <sub>OXA-23</sub>	<i>aadB</i> -like, <i>aph(3'')-Vla</i> -like, <i>aph(3'')-Vlb</i> -like, <i>strA</i> -like, <i>strB</i> -like	<i>sul2</i>			<i>tet(B)</i>		<i>mph(E)</i> , <i>msr(E)</i>	S83-L	S80-L
N84	<i>bla</i> <sub>OXA-317</sub> , <i>bla</i> <sub>PER-1</sub> , <i>bla</i> <sub>OXA-23</sub>	<i>aadB</i> -like, <i>aph(3'')-Vla</i> -like, <i>aph(3'')-Vlb</i> , <i>strA</i> -like, <i>strB</i>	<i>sul2</i>			<i>tet(B)</i>		<i>mph(E)</i> , <i>msr(E)</i>	S83-L	S80-L
N51	<i>bla</i> <sub>OXA-317</sub> , <i>bla</i> <sub>PER-1</sub> , <i>bla</i> <sub>TEM-1D</sub>	<i>aadB</i> -like, <i>aph(3'')-Vla</i> -like, <i>strA</i> -like, <i>strB</i> -like	<i>sul2</i>			<i>tet(B)</i>		<i>mph(E)</i> , <i>msr(E)</i>	S83-L	S80-L
N54	<i>bla</i> <sub>OXA-1*</sub> , <i>bla</i> <sub>OXA-66*</sub> , <i>bla</i> <sub>TEM-1D</sub>	<i>aac(6'')-II</i> -like, <i>aph(3'')-Vla</i> -like, <i>strA</i> , <i>strB</i>	<i>sul1</i> , <i>sul2</i>			<i>tet(B)</i> -like			S83-L	S80-L
N57	<i>bla</i> <sub>NDM-1*</sub> , <i>bla</i> <sub>OXA-66*</sub> , <i>bla</i> <sub>TEM-1D</sub>	<i>aac(6'')-II</i> -like, <i>aph(3'')-Vla</i> -like, <i>strA</i> , <i>strB</i>	<i>sul1</i> , <i>sul2</i>			<i>tet(B)</i> -like			S83-L	S80-L
N99	<i>bla</i> <sub>OXA-1*</sub> , <i>bla</i> <sub>OXA-66*</sub> , <i>bla</i> <sub>TEM-1D</sub>	<i>aac(6'')-II</i> -like, <i>aph(3'')-Vla</i> -like, <i>strA</i> , <i>strB</i>	<i>sul1</i> , <i>sul2</i>			<i>tet(B)</i> -like			S83-L	S80-L
N27	<i>bla</i> <sub>OXA-23*</sub> , <i>bla</i> <sub>TEM-1D</sub>	<i>aph(3'')-Ic</i> -like, <i>aph(3'')-Vla</i> -like	<i>sul1</i> , <i>sul2</i>			<i>tet(B)</i> -like		<i>mph(E)</i> , <i>msr(E)</i>	S83-L/E87-V	S80-L
N25	<i>bla</i> <sub>OXA-23*</sub> , <i>bla</i> <sub>OXA-66*</sub> , <i>bla</i> <sub>TEM-1D</sub>	<i>aph(3'')-Ic</i> -like, <i>strA</i> , <i>strB</i>	<i>sul1</i> , <i>sul2</i>			<i>tet(B)</i> -like		<i>mph(E)</i> , <i>msr(E)</i>	S83-L	S80-L
N25	<i>bla</i> <sub>OXA-66*</sub> , <i>bla</i> <sub>TEM-1D</sub>	<i>aac(6'')-II</i> -like, <i>strA</i> , <i>strB</i>	<i>sul1</i> , <i>sul2</i>			<i>tet(B)</i> -like		<i>mph(E)</i> , <i>msr(E)</i>	S83-L	S80-L

<sup>a</sup>Isolates are ordered in accordance with Table 1.

<sup>b</sup>FQ/AMG, fluoroquinolone/aminoglycoside.

<sup>c</sup>TET, tetracycline.

<sup>d</sup>TMP, trimethoprim.

The results were averaged, normalized, and plotted against the reference strain *A. baumannii* (DSM 30008) (14). The results showing the growth curves are summarized in Fig. S1 to S5 in the supplemental material, where the isolates are grouped according to their IC/ST. Most isolates grew at a similar rate as the control strain for the first 10 h. However, after 10 h, differences emerged. For example, five of the six IC2 isolates all grew to higher densities at 16 h, suggesting these isolates have an increased fitness (Fig. S2). Furthermore, in TC-D, both N53 (carbapenem resistant) and N45 (carbapenem susceptible) grew to a higher density than the control, suggesting that the acquisition of carbapenem resistance had no effect on fitness as measured by growth rates (Fig. S5). Kumar et al. showed that CRAB were fitter than carbapenem-susceptible isolates (15). Interestingly, some isolates that were identical by cgMLST and their resistomes exhibited different growth rates; TC-C comprised three isolates, with N19 growing to a lower density, N44 to a higher density, and N20 to the same density as the control (Fig. S1). Taken together, these data suggest that growth rates, while variable between isolates, did not necessarily correlate with clustering, and the acquisition of a carbapenemase was not necessarily detrimental as has sometimes been suggested (16, 17).

Our study demonstrates persistently high rates of CRAB in three hospitals in Tripoli, Libya, a country still in the midst of a decade-long civil war. Rather than a single clone predominating, our results demonstrate, more worryingly, that a diverse population of *A. baumannii* have acquired carbapenem resistance genes at no fitness cost and are readily spread between hospitals. The lack of fitness cost may explain why no single clone has outcompeted the others. Further research assessing hospital practices, antibiotic use, and infection control protocols, combined with country-level and patient-level data such as periods of active combat and care trajectories of patients as well as isolates from other major cities, would likely shed more light on the underlying dynamics affecting the spread of polyclonal CRAB in Libya.

**Data availability.** Raw sequencing reads were submitted to the European Nucleotide Archive under BioProject accession number [PRJEB42042](https://www.ebi.ac.uk/bioproject/12042).

## SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

**SUPPLEMENTAL FILE 1**, PDF file, 0.3 MB.

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