

Phenotypic and Genotypic Diversity of *Staphylococcus aureus* Isolated from Livestock and Human

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Abstract: *Staphylococcus aureus* is a zoonotic well versed bacterial species. It produces plethora of infections along various localization sites all over the body equally in animals and humans. So the study directed to investigate the extent of *S. aureus* in wound lesions of animals and humans, probe their antimicrobial susceptibility behavior against antimicrobial agents of veterinary and human medicine concern and determine the relatedness of the recovered isolates comparing their genetic assortment by RAPD fingerprinting. *S. aureus* was recovered from wounds of both animals and humans. In animals the highest *S. aureus* recovery rate was noted in equine surgical wounds and in relation to animal species also equine showed the topmost recovery rate followed by bovine and lasted by ovine wound lesions. Antimicrobial susceptibility testing revealed high rate of methicillin resistance, clindamycin resistance and multidrug resistance in *S. aureus* of both animal and human origins. Vancomycin resistance appeared in *S. aureus* of animal but not in those of human origin. Necessitate actions are needed to identify causes behind vancomycin resistant *S. aureus* in animals for antimicrobial stewardship and prevent jump to humans. RAPD revealed the probability of *S. aureus* broadcast back and forth between animals and humans in addition to the opportunity of wound multi-infection with more than one strain of *S. aureus*.

Key words: MRSA • RAPD • *Staphylococcus aureus* • Vancomycin • VRSA

INTRODUCTION

Staphylococcus aureus is a well versed bacterial species that has bad repute. It boasts a diverse array of virulence factors [1] produces plethora of infections along various localization sites all over the body equally in animals and humans. In animals, *S. aureus* elicits mastitis [2], bacteremia, pneumonia, septic arthritis, omphalophlebitis and osteomyelitis [3]. Above and beyond, *S. aureus* itself and its toxins could threaten human's health eliciting endocarditis [4], pneumonia [5], toxic shock syndrome and metastatic abscesses [6] and food borne intoxication [7].

What is more, *S. aureus* commonly allied with skin infections in animals [8] and humans [9]. Careless attention to *S. aureus* infected wounds could exaggerate the already existent skin lesion or even it could pass through blood or lymph to the internal organs causing various obsessive illnesses; the waited scenario since the wound befallen.

Contact of diseased animals and healthy humans and vice versa, allows not only *S. aureus* transmission back and forth between animals and humans [10] but also allows genetic determinants broadcast amongst *S. aureus* of both origins including resistance genes [11] as it grants the transfer of plasmids mediated resistance to a range

of drugs including vancomycin [12] that escalate the burden of multi-drug resistance; the emerging worldwide crisis.

Scholars usually focus on the transmission of *S. aureus* from animals to humans [13] that seemed a usual event. But other researchers [10] adopted and documented the jump of human *S. aureus* to bovine. Subsequently, epidemiological declaration of zoonotic bacterial species alike *S. aureus* needs simple rapid technique to put down the real situation of such condition in step to control spread of zoonotic bacteria between animals and humans.

Random Amplified Polymorphic DNA (RAPD) is a polymerase chain reaction uses single short primers with arbitrary nucleotide sequences. The profiles obtained after electrophoretic separation of the amplification products can be used to fingerprint strains of varies prokaryotic and eukaryotic species [14].

So the current study directed to look at the extent of *S. aureus* in animals and humans suffered wound lesions, probe their antimicrobial resistance behavior and investigate their genetic assortment by RAPD fingerprinting in a trial for epidemiological trace backing.

MATERIALS AND METHODS

Samples: Wound swabs were aseptically collected from 99 and 65 wounds in different animal species and humans (animal contacts) respectively at Fayoum Governorate during the period from January 2013 up to December 2013 (Table 1). Swabs were transferred to laboratory in ice-cooled container and processed directly on arrival. Lesions were identified as abscesses, surgical and non-surgical wounds.

Isolation and Identification of *S. aureus*: Isolation and identification of *S. aureus* was carried out according to Collee *et al.* [15].

Antimicrobial Sensitivity Testing:

Disk Diffusion Test: Disk diffusion test was performed and judged in accordance to Clinical and Laboratory Standards Institute (CLSI) [16]. *S. aureus* isolates were

inspected for their susceptibility behavior against various antimicrobial agents used in veterinary and human medicine practices; amoxicillin/clavulanic acid (AMC 20/10), cefoxitin (FOX 30 µg), ciprofloxacin (CIP 5 µg), clindamycin (DA 2 µg), doxycycline (DO 30 µg), gentamicin (CN 10 µg), penicillin G (P 10 U), rifampicin (RD 5 µg) and spectinomycin (SH 100 µg). Cefoxitin resistance indicated methicillin resistant phenotype according to CLSI [16].

Minimal Inhibitory Concentration (MIC) Test: Susceptibility behavior of *S. aureus* isolates under test against vancomycin was inspected using agar dilution method in concordance to CLSI [16].

PCR-RAPD Analysis: Twelve *S. aureus* isolates representing various antimicrobial susceptibility patterns were investigated. Six human *S. aureus* isolates numbered *S. aureus* 1 to *S. aureus* 6 (four representing four wounded patients and two from a single patient numbered *S. aureus* 3 and *S. aureus* 4). Additional six animal *S. aureus* isolates numbered *S. aureus* 7 to *S. aureus* 12 (four from four animals and two from single wounded animal numbered *S. aureus* 9 and *S. aureus* 12). All isolates were analyzed for likeness degree using RAPD technique as previously described by Byun *et al.* [17]. Primers used were OPA 03: 5' -AGT CAG CCA C- 3', OPA 04: 5' -AAT CGG GCT G- 3', OPA 05: 5' -AGG GGT CTT G- 3' and OPA 06: 5' -GGT CCC TGA C- 3 which were manufactured by Invitrogen, UK.

Firstly, *S. aureus* isolates were cultivated in brain heart infusion broth at 37°C for 18 h. Bacterial DNA was extracted according to the manufacturer QIAGEN extraction kit. Extracted DNA was verified for concentration and purification by spectrophotometer. Amplification was carried out in a total reaction of 50 µL (2X PCR master mix, 25 µL; primers, 20 pmol; template DNA, 30 ng and DNase free water when needed). Amplification condition was done with little modification of that performed by Byun *et al.* [17] to meet the variation in the thermal cycler conditions. Initial denaturation at 95°C for 5 minutes, 40 cycles (denaturation at 95°C for 30 seconds, annealing at 25°C for 60 seconds and extension

Table 1: Samples collected from animals and humans

		Surgical wounds	Non-surgical wounds	Abscesses	Total
Animals	Bovine	2	21	14	37
	Ovine	2	16	19	37
	Equine	5	13	7	25
	Total	9	50	40	99
Humans	Animal contacts	17	23	25	65

at 72°C for 1.5 minutes) followed by final extension at 72°C for 10 minutes to ensure complete elongation of all amplified products.

Amplification products were run in 1.2% agarose gel containing ethidium bromide 0.5 µg/mL. Ladder, 3000bp (begin with 100 bp and increased 100 bp in each band till 1000 bp band then follow by 1500 and 3000 bp bands respectively) was used as a DNA fragment size marker and photographed in UV light.

RAPD analysis and dendrograms construction were performed using GelQuest [18] and ClusterVis [19] software. Similarity of *S. aureus* isolates with 70% or more refereed the probability of the same origin [17].

RAPD analysis discriminatory power (D) was calculated for each primer alone and for the sum results in accordance to Hunter [20] using the following formula:

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^s x_j(x_j - 1)$$

D: Discriminatory power

N: The number of unrelated strains

S: Number of groups

x_j: Number of members falling in the jth type

RESULTS

Isolation and Identification: *S. aureus* was recovered from all examined wounded animal species and humans. In animals, the highest recovery rate was in equine surgical wounds (80%). In relation to animal species, the highest recovery rate was noted in equine (24%) followed by bovine and lasted with ovine. Two *S. aureus* isolates were recovered from the same surgical wounded horse.

In relation to the wound type, abscesses showed the highest *S. aureus* recovery rate while, surgical wounds showed the lowest *S. aureus* recovery rate. Of note, two isolates were recovered from the same human non-surgical wound type.

Moreover, the overall *S. aureus* recovery rate in humans exceeded that in animals, 20 versus 11.1% respectively (Table 2).

Antimicrobial Sensitivity Testing:

Disk Diffusion Test: Disk diffusion susceptibility testing revealed non-susceptible patterns (i.e. resistant and intermediate susceptibility) of *S. aureus* of animal origin exceeded that of human origin with many antimicrobial agents including amoxicillin-clavulanic acid, clindamycin, spectinomycin, doxycycline, rifampicin and ciprofloxacin. On the other hand, non-susceptible patterns of *S. aureus* of human origin exceeded that of animal origin when tested against cefoxitin and gentamicin. Both staphylococcal isolates of animal and human origins showed 100% non-susceptible pattern against penicillin (Table 3).

Minimal Inhibitory Concentration (MIC) Test: Two *S. aureus* isolates (16.7%) of animal origin were resistant to vancomycin while all *S. aureus* isolates of human origin were vancomycin-susceptible (100%).

Within the milieu of the recorded high resistance patterns, MDR was 100 and 92.9% for staphylococcal isolates of both animal and human origins respectively.

PCR-RAPD Analysis: Three primers (OPA 03, OPA 04, OPA 05 but not OPA 06) out of the used four primers in RAPD analysis yielded amplicons with only 11 *S. aureus*.

Table 2: *S. aureus* recovery rates from wounded animals and humans

		Surgical wounds		Non-surgical wounds		Abscesses		Total	
		+	%	+	%	+	%	+	%
Animal species	Bovine	0 (2)	0.0	0 (21)	0.0	3 (14)	21.4	3(37)	8.1
	Ovine	0 (2)	0.0	0 (16)	0.0	2 (19)	10.5	2(37)	5.4
	Equine	*4 (5)	80.0	2 (13)	15.4	0 (7)	0.0	6(25)	24.0
	Total	4 (9)	44.4	2 (50)	4.0	5 (40)	12.5	11(99)	11.1
Humans	Animal contacts	2 (17)	11.8	#5 (23)	21.7	6 (25)	24.0	13 (65)	20.0

+: number of *S. aureus* positive casesno.: number of examined wounds

#: percentage of positive cases for isolation of *S. aureus* in relation to each lesion examined number

*: Four surgical wound lesions from equine were positive for *S. aureus* isolation and one of them reproduced two different strains of *S. aureus* (i.e. five isolates were recovered from four animals).

#: Five humans suffered non-surgical wound were positive for *S. aureus* isolation and one of them reproduced two different *S. aureus* strains (i.e. six isolates recovered from five humans).

Table 3: Susceptibility patterns of *S. aureus* isolated from both animal and human origins

	<i>S. aureus</i> of animal origin no=12				<i>S. aureus</i> of human origin no= 14			
	Susceptible		*Non-susceptible		Susceptible		*Non-susceptible	
	No.	%	No.	%	No.	%	No.	%
Amoxicillin-clavulanic	0	0.0	12	100.0	2	14.3	12	85.7
Cefoxitin	5	41.7	7	58.3	5	35.7	9	64.3
Ciprofloxacin	10	83.3	2	16.7	12	85.7	2	14.3
Clindamycin	2	16.7	10	83.3	5	35.7	9	64.3
Doxycycline	3	25.0	9	75.0	10	71.4	4	28.6
Gentamicin	9	75.5	3	25.0	10	71.4	4	28.6
Penicillin	0	0.0	12	100.0	0	0.0	14	0.0
Rifampicin	6	50.0	6	50.0	12	85.7	2	14.3
Spectinomycin	2	16.7	10	83.3	11	78.6	3	21.4

*: Non-susceptible means resistant and/or intermediate behavior of *S. aureus* against the tested antimicrobial agents.

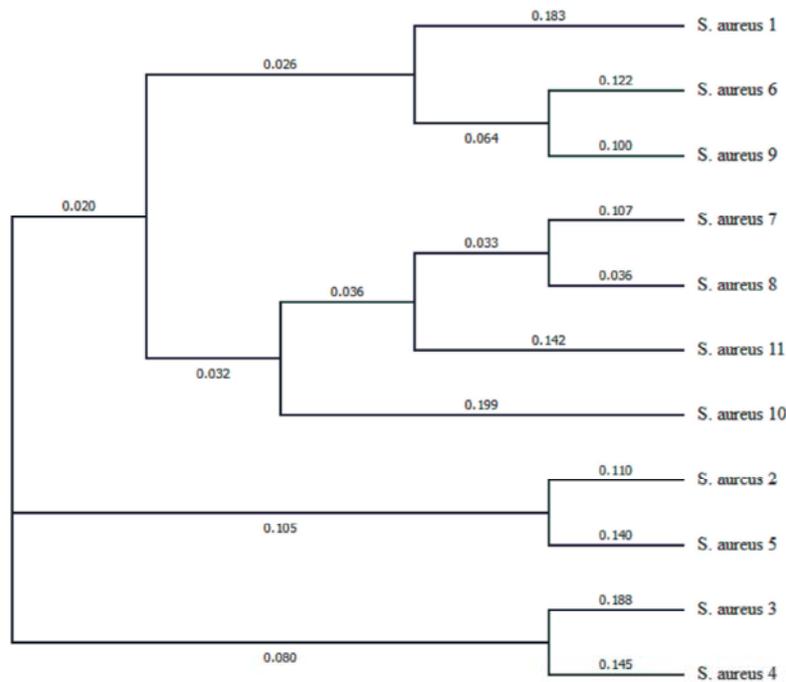


Fig 1: Dendrogram of 11 *S. aureus* (1-6 of human origin and 7-11 from animal origin) using OPA 03 primer reveals at least 70% similarity among the tested *S. aureus* isolates.

Since *S. aureus* 12, one of two *S. aureus* isolates recovered from the same horse failed yielding any amplicon with the tested primers.

Figures (1, 2 and 3) illustrate the similarity degrees of the different primers across the 11 *S. aureus* reacted with RAPD analysis. OPA 03 primer (Figure 1) failed to discriminate *S. aureus* of animal origin from those of human origin (D= 0). OPA 04 (Figure 2) showed better discriminatory power (D= 0.69) but was unable to differentiate animal from human *S. aureus*. It discriminated the tested 11 *S. aureus* into three

groups: one of them contained five isolates, three human *S. aureus* and two animal *S. aureus*; the second contained four *S. aureus* encountered one of animal origin and three of human origin; while the last group contained two *S. aureus* of animal origin only. Higher discriminatory power (D= 0.89) was realized by OPA 05 primer (Figure 3) which distinguished the 11 tested *S. aureus* into six groups, two of them contained animal and human *S. aureus*, two contained isolates of animal origin only and the last two contained human *S. aureus* only.

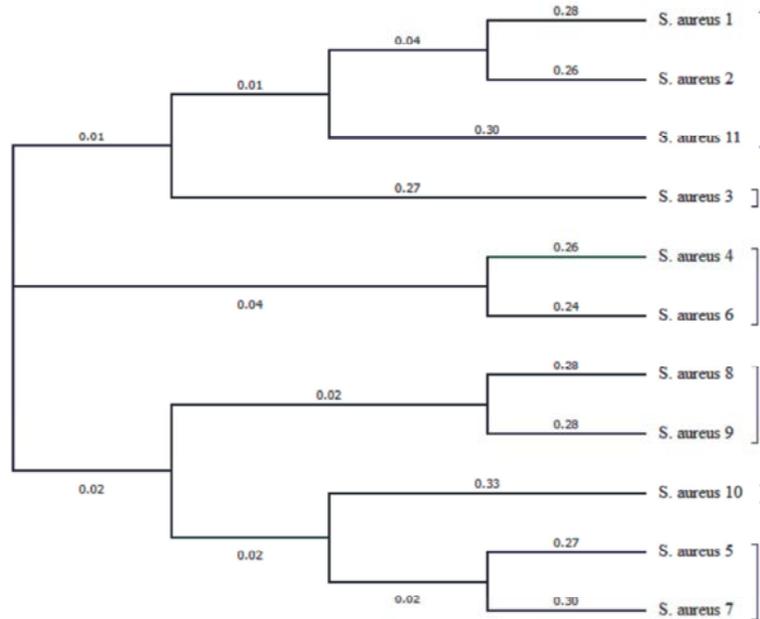


Fig 2: Dendrogram of 11 *S. aureus* (1-6 of human origin and 7-11 of animal origin) using OPA 04 primer discriminates the tested isolates into three groups two of them including isolates of both animal and human origins.

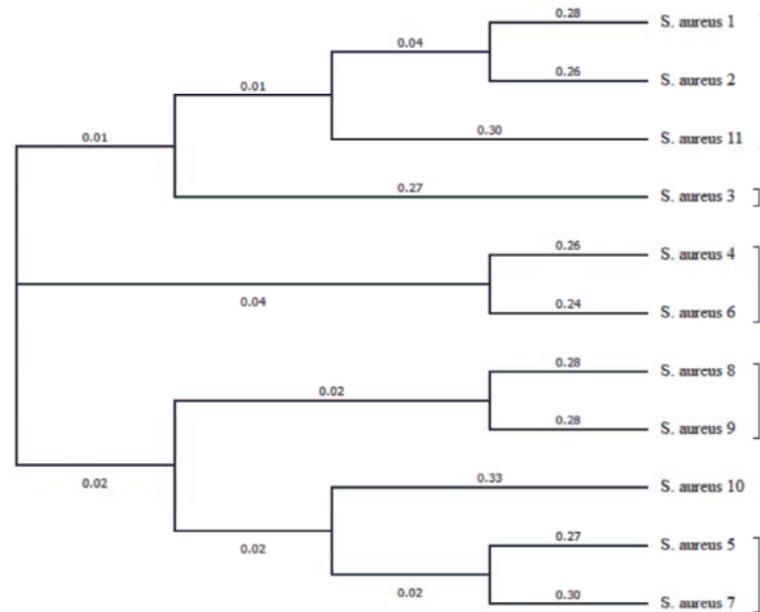


Fig 3: Dendrogram of 11 *S. aureus* (1-6 of human origin and 7-11 of animal origin) using OPA 05 primer categorizes the tested isolates into six groups two of them including isolates of both animal and human origins.

The utmost discriminatory power ($D= 0.93$) was achieved by sum results of OPA 04 and OPA 05 primers discriminated the tested 11 *S. aureus* into eight groups, two out of them contained animal and human staphylococcal isolates. Group 1 contained three staphylococcal isolates (*S. aureus* 1, 2 from humans and *S. aureus* 11 from equine), group 2 contained two

staphylococcal isolates (*S. aureus* 5 from human and *S. aureus* 7 from bovine), while the other six groups each contained single staphylococcal isolate. What is more, OPA 04 and OPA 05 primers grouped two *S. aureus* isolates (*S. aureus* 3 and *S. aureus* 4) recovered from the same human patient into two different groups.

DISCUSSION

Microbiological analysis of wound lesions documented the recovery of *S. aureus* from both wounded animals and humans that imitate the environmental microbial load [21] with noticeable difference among different wound types in both wounded animals and humans. Additionally, in animals the prevalence varied with animal species, the highest recovery rate was associated with surgical wounded equine 80% (4/5) followed by abscesses in bovine 21.4% (3/14) and last of all was abscesses in ovine 10.5% (2/19), moreover, *S. aureus* could not be isolated from equine abscess samples. Similar finding was reported by Ibrahim [22] who isolated *S. aureus* from ovine but not from equine abscesses.

In humans, *S. aureus* recovery rate varied in relation to lesion type, the highest prevalence was noticed in abscesses (24%), which compares closely with the 21% finding of Lamy *et al.* [23]. Additionally, recovery rate of *S. aureus* was 11.7% in surgical wounded humans that less closely matches the 25% reported by Shahane *et al.* [24]. The lower recovery rate of *S. aureus* reported in human surgical wounds than that noted in equine surgical wounds could be attributed to perioperative empirical antibiotic prophylaxis in human surgery which prevents surgical site infections [25].

Antimicrobial susceptibility testing of the recovered *S. aureus* against drugs of veterinary and human medicine concern showed worrisome results. Beside the high rate of methicillin resistance and MDR resistance reported in *S. aureus* of both animal and human origins, vancomycin resistance appeared in *S. aureus* isolates of animal origin (16.7%) but not in those of human origin.

Haaber *et al.* [26] recently noted that *in vitro* exposure of *S. aureus* to colistin sulphate -the widely used antibiotic in veterinary medicine- accompanied by reversible gene expression changes analogous to those resulting in mutations that yield stably inherited reduction of vancomycin sensitivity and was persisted only as long as colistin was present. Nonetheless the *in vivo* exposure might result in irreversible changes in gene expression. Another assumed cause, livestock are considered as a reservoir of vancomycin resistant *Enterococcus faecalis* [27], moreover it is known about *S. aureus* the ability to secrete an *E. faecalis*-specific sex pheromone that trigger genes transfer including vancomycin resistance gene which was previously documented in laboratory by Noble *et al.* [28].

Antimicrobial susceptibility testing results could answer the puzzled question in the field of microbiology; does antimicrobial resistance being zoonosis or humenosis? Our results could assume that antimicrobial resistance being zoonosis rather than humanosis.

Biochemical identification of *S. aureus* fails to tell apart *S. aureus* recovered from animals and/or humans. Additionally, antibiogram biotyping does not work all times [29] that disallow discrimination of *S. aureus* recovered from different species of livestock and humans. RAPD fingerprinting previously showed added values in molecular epidemiology of *S. aureus* trace backing [30].

RAPD assay uses short primers with an arbitrary sequence to amplify genomic DNA in PCR reaction. Primers anneal with chromosomal sequences at various sites of different strains and so forth produce different amplification products. These products can be separated by gel electrophoresis to produce fingerprints or patterns characteristic of different epidemiological types as described by Tambic *et al.* [31].

In the present study, RAPD analysis was performed using four primers (OPA 03, OPA 04, OPA 05 and OPA 06) that were recommended by Byun *et al.* [17]. OPA 06 primer did not work at all and this may be due to its high discriminatory power described by the authors so it may be worked with their regional staphylococcal strains. Moreover, out of the 12 tested *S. aureus* one of the six animal *S. aureus* did not give any bands with the three worked primers.

OPA 03, OPA 04 and OPA 05 primers showed sundry results in RAPD fingerprints of the 11 *S. aureus* isolates noticed by computer analysis using GelQuest version 3.2.1 software [18]. Judging the strains similarity in relation to 70% likeness [17], OPA 03 (Figure 1) clustered all *S. aureus* in single group (D= 0). OPA 04 resulted in three clusters (D= 0.69), one of them contained five isolates, three human *S. aureus* and two animal *S. aureus*; the second contained four *S. aureus* encountered one of animal origin and three of human origin; while the last group contained two *S. aureus* of animal origin only (Figure 2). Higher discriminatory power (D= 0.89) was realized by OPA 05 primer which clustered the 11 tested *S. aureus* into six groups, two of them contained animal and human *S. aureus*, two contained isolates of animal origin only and the last two contained human *S. aureus* only (Figure 3).

The utmost discriminatory power (D= 0.93) was achieved by sum results of OPA 04 and OPA 05 primers discriminated the tested 11 *S. aureus* into eight groups, two out of them contained animal and human staphylococcal isolates. Group 1 contained three

staphylococcal isolates (*S. aureus* 1, 2 from humans and *S. aureus* 11 from equine) and this tie to Weese [3] results that documented the jump of equine staphylococcal strain to human wound. Group 2 contained two staphylococcal isolates (*S. aureus* 5 from human and *S. aureus* 7 from bovine) which concede with the results of Köck *et al.* [13] and Pereira *et al.* [32] who reported the same RAPD profile of animal and human *S. aureus*. On the other hand, the other six groups each contained single staphylococcal isolate indicates genetic heterogeneity and the circulation of many *S. aureus* clones in the area under study. Of note, OPA 04 and OPA 05 primers grouped two *S. aureus* isolates (*S. aureus* 3 and *S. aureus* 4) recovered from the same human patient into two different groups i.e. co-infection of the same wound with two genotypically different strains [17]. The results signposted RAPD to be a valuable tool for molecular epidemiology, tracing the sources of *S. aureus* infection at inter-hosts or intra-host level using fit set of primers.

This study concluded accompany of *S. aureus* with wounded animals and humans, reported high rate of methicillin resistance, clindamycin resistance and multidrug resistance in *S. aureus* of both animal and human origins. Vancomycin resistance appeared in *S. aureus* of animal but not in those of human origin. Necessitate actions are needed to identify causes behind vancomycin resistant *S. aureus* in animals for antimicrobial stewardship and prevent *S. aureus* jump to humans that could reflect positively in human health. Finally, the results signposted RAPD to be a valuable tool for molecular epidemiology, tracing the sources of *S. aureus* infection at inter-hosts or intra-host level using fit set of primers.

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