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Sugar-fermenting flora mask *Vibrio cholerae* in Kligler iron agar

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Cholera is a severe diarrheal disease caused by *Vibrio (V.) cholerae*, a Gram-negative, motile bacterium. The pathogen colonizes the small intestine and produces an exotoxin that disrupts sodium transport, leading to massive fluid loss, dehydration, and potentially death if untreated[1,2].

Kligler iron agar is used to differentiate enteric bacteria and can serve as a short-term transport medium for suspected *V. cholerae*. It contains glucose, lactose, and phenol red as a pH indicator. Glucose fermentation turns the butt yellow (anaerobic), while lactose fermentation turns the whole medium yellow due to sustained acid production[3].

In the current study, *V. cholera* O1 serotype ogawa were isolated from eight Iraqi patients in Al-Shomali General Hospital, Babylon city, Iraq. Isolates were obtained from watery diarrheal stool and then cultured in peptone water for 8 h, followed by overnight incubation on blood agar, MacConkey agar and TCBS agar (HI Media, India) to differentiate between different species. They were biochemically tested using oxidase, indole, urease, simmons citrate, Kligler iron agar, Gram staining, string test and antisera polyvalent ogawa and inaba (Hardy Diagnostics, USA). Isolates were confirmed by VITIK-2[4]. Eight from all these microbes including *Escherichia (E.) coli*, *Klebsiella* spp., *Enterococcus* spp., *Staphylococcus aureus*, *Pseudomanas* spp., *Bacillius* spp. and *Candida* spp. and *V. cholera* isolates were obtained from watery diarrheal stool then cultured in peptone water for 8 h. *V. cholerae* was cultured together with an equal quantity of each bacterial species in Kligler iron agar for 24 h by taking a single colony of *V. cholerae* and a single colony of the other bacteria. The cultures were then incubated on blood agar for 24 h to observe the growth of *V. cholerae* compared to the other bacteria by direct colony counting for growth rate comparison.

For statistical analysis, SPSS software 26 (SPSS Inc., Chicago, USA) was used. *Chi*-square test was used to represent the data with *P* value<0.05 considered significant.

The results of this study showed the growth rate comparison between total growth rate of all bacterial isolates (75%) and *V. cholera* (25%) with a significant difference ($P<0.0001$) indicating that other bacteria inhibit the growth of *V. cholera* while the proposed rate of growth should be 50% for *V. cholera* and each other bacterium.

A growth rate comparison between *V. cholerae* and various microbial isolates revealed significantly lower growth of *V. cholerae* on blood agar after co-culture in Kligler iron agar. The relative growth percentages of *V. cholerae* compared to other species were as follows: 0% *V. cholerae vs.* 100% *Candida* spp. ($P<0.0001$), 1.5% *V. cholerae vs.* 98.5% *Klebsiella* spp. ($P<0.0001$), 4.0% *V. cholerae vs.* 96.0% *E. coli* ($P<0.0001$), 30.0% *V. cholerae vs.* 70.0% *Bacillus* spp. ($P<0.0001$), 57.5% *Enterococcus* spp. *vs.* 42.5% *V. cholerae* ($P=0.0040$), and 52.5% *Staphylococcus aureus vs.* 47.5% *V. cholera* ($P=0.0400$). No significant association between *V. cholera* (50.0%) and *Pseudomonas* spp. (50.0%) was found ($P=1.0000$, Table 1).

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Table 1. Growth rate comparison between each bacterial isolates and *Vibrio cholera*.

Bacterial isolates	Type	No.	Percentage of growth	*P*
Enterococcus spp.	Bacteria	8	57.5%	0.0040
	Cholera	8	42.5%	
Staphylococcus aureus	Bacteria	8	52.5%	0.0400
	Cholera	8	47.5%	
Klebsialla spp.	Bacteria	8	98.5%	< 0.0001
	Cholera	8	1.5%	
Escherichia coli	Bacteria	8	96.0%	< 0.0001
	Cholera	8	4.0%	
Pseudomonas spp.	Bacteria	8	50.0%	1.0000
	Cholera	8	50.0%	
Bacillus spp.	Bacteria	8	70.0%	< 0.0001
	Cholera	8	30.0%	
Candida spp.	Bacteria	8	100.0%	< 0.0001
	Cholera	8	0.0%	

As we know *V. cholera* prefers an alkaline environment and is inhibited in acidic environment. This study concluded that when *V. cholera* mixed with bacteria capable of lowering the pH to a high acidic level in Kligler iron agar, which leads to the killing or slowed growth of *V. cholera*, depending on these ability of bacteria to acidify the environment. Another study also discussed the effects of acid shock on *V. cholera* that mentioned the exposure of *V. cholera* to mild acid shock (pH 5.5) slightly reduced cell viability[5]. Another study also revealed when bacteria were subjected to low acidic pH of the gastric environment, they might enter a viable but non-culturable state for survival[6].

In the current study, bacteria that ferment both glucose and lactose produced low pH that leads to strong inhibition of *V. cholera* growth as observed with *E. coli* and *Klebsiella*. Bacteria that ferment only glucose moderately inhibit *V. cholerae* growth. In contrast, bacteria that neither ferment glucose nor lactose, such as *Pseudomonas aeruginosa*, have no effect on *V. cholerae* growth. *Candida albicans* tends to ferment sugar rapidly, fully inhibiting the growth of *V. cholerae*.

In conclusion, bacteria that ferment sugars or utilize sugars and turn pH acidic in Kligler iron agar can kill or inhibit *V. cholera*. Therefore, when *V. cholera* contaminated with another fermentable normal flora in Kligler iron agar that sent by terminal laboratories for confirming the result in central laboratory, *V. cholera* may be killed or inhibited by high acidity due to sugar fermentation in Kligler iron agar.

Conflict of interest statement

The author claim there is no conflict of interest.

Authors' contributions

Alhusayni AA conceptualized and developed the study design, reviewed the literatures, took part in proposal development, led the data collection and data entry, and took part in data analysis and interpretation, manuscript writing and review. Al-Khikani FHO also conceptualized and developed the study design, participated in data analysis and interpretation, engaged in proposal development, and contributed to manuscript preparation and review. All authors have read and approved the final version of the manuscript.

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Erratum: Fecal shedding and transmission of vaccine–derived poliovirus: A systematic review and meta–analysis
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The authors would like to correct an error in Results. The original sentence reads: "No statistical significance is reported (solicited). The adverse events was observed between the two doses (*OR*: 1.87, 95%*CI*: 0.15-22.66, *P*=0.14; I^2= 70.33%) (Figure 2E), whereas statistical significance in unreported (unsolicited) adverse events was observed between the two doses (*OR*: 1.05, 95%*CI*: 0.98-1.13, *P*=0.001, I^2 = 0%) (Figure 2F)". The sentence should have read: No statistically significant difference was observed between the two doses for either solicited adverse events (*OR*: 1.87, 95%*CI*: 0.15-22.66, *P*=0.14; I^2= 70.33%) (Figure 2E) or unsolicited adverse events (*OR*: 1.05, 95%*CI*: 0.98–1.13, *P*=0.161, I^2=0%) (Figure 2F)." The error does not affect the conclusion of the study. The authors apologize for the error and the inconvenience it might have caused to readers.