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Comparison Between Conventional and Molecular Identification of *Vibrio Cholerae* Isolated From Human and Birds

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Abstract

THIS Study aimed to comparison between diagnostic methods to evaluated the prevalence of Vibrio *cholerae* .A total of 90 samples include 40 birds faeces were collected from bird farms and birds breeding stores for Ducks, chickens, Pigeons, Pet birds and 50 hand swab from owners of birds (Males and Females) .collection from different places Al-hurya city, Abu Ghraib city, AL-Yarmouk area ,University district area in Baghdad Province, at the period of February 2021 to February 2022, in order to evaluate the routine laboratory diagnostic procedures in the diagnosis of *V. cholerae* isolates and compared them with molecular methods as Polymerase chain reaction (PCR).*V.cholerae* has been isolated and identified by using culturing method in addition to biochemical tests, Vitek2 system. The results showed that in culturing method percentage of isolation (43.33%)from birds feces, and (33.33%) in hand swabs,in biochemical tests(33.33%) in birds feces, and (10%)from hand swabs in Vitek2 system (6.7%)from each birds feces and hand swabs. PCR technique was used to detect sodB Gene encoding to outer membrane protein of *V. cholerae* ,on the PCR results, the rate of *V. cholerae* isolation from birds feces samples was 23.3 %. and 6.66% from hand swab samples,in Comparison PCR results showed that there were high sensitivity 100% with cultural, biochemical test , while 44% with Vitek2 system respectively.in presnt study found that diagnosis of *V.cholerae* by routine diagnostic may be misidentifications and using of PCR more faster, accurate ,sensitive and specific for correct identification

Keywords:. *V. cholerae*, Vitek 2system, *sodB* Gene, bird owners.

Introduction

V. cholerae is a facultative, gram-negative, motile that secretes a cholera toxin causing acute diarrheal disease called cholera a health problem with Diarrhea watery stool leading to dehydration and deaths of patients without medicine [1-4]. Cholera results from consuming food or water contaminated with *V.cholerae* it generates an enterotoxin resulting in painless, septicemia vomiting in the host within a few hours to two or three days of ingestion. It also causes the small intestine to get colonized with harmful bacteria watery diarrhea severe dehydration sunken eyes, cold skin, decreased skin elasticity, and wrinkling of the hands and feet. Dehydration can cause the skin to turn bluish. and even death if treatment is delayed [5-8]. Cholera in humans has been connected to birds after close contact with avian species, which may increase the risk of infections. Although that infection can transmit from birds

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to domestic animals indirectly, drinking water contaminated with excrement or contacting inanimate things that have been exposed to bird secretions or droppings are two ways that humans can get the disease [9-12]. Methods for identifying *V.cholerae*, like culturing and biochemical testing, are laborious and time-consuming. However, Molecular techniques be considered as important tools in monitoring strains of *V. cholerae* nucleic acid-based molecular biological techniques are common, and the use of PCR tools are suitable for this task because of their speed, specificity, and high accuracy, as described by [13-16]. This study aimed to identify *V.cholerae* isolates from human and birds using PCR with *sodb* gene and comparison with routine laboratory diagnostic procedures for accurate characterization of *V.choleare*

Material and Methods

Ethics approval

The present work has received approval from the College of Veterinary Medicine at the University of Baghdad. The researcher collected samples with the earlier mentioned ethical standards.

Processing of Samples

Ninety samples including from (40 birds feces and 50 hand swabs from owners) were collected in sterile containers in Baghdad province, during the period from 2/2021 to 2/2022.

Isolates Identification

The samples were Culturing in alkaline peptone water (APW).(Himedia/India) and incubated at 37ºC for 24hrs.then a loopful from APW streaked on to Thiosulphate Citrate Bile salt Sucrose agar (TCBS) and incubated at 37ºC. Suspected colonies of Vibrio were picked up and streaked on hiCrome vibrio agar (Himedia/India) and MacConkey agar [17].

Suspected Vibrio colonies were transferred to nutrient agar and incubated at 37C for 24 hours for further identification. Microscopic examination and the following biochemical tests: triple sugar iron agar medium (TSI), sulfide indol motility, oxidase, urease production Methyl red Voges proskauer, Simmons Citrate and string test VITEK2 compact system was used to confirmed the isolates was done according to manufacturer company using gram negative cards [18].

Molecular Identification

According to DNA extraction protocol of company instructions(Easy Pure® Bacteria Genomic DNA Kit) DNA extraction of isolated colony of *V. cholerae* was done . The PCR-amplification done by using assay based on repetitive sequence *sodB* gene of *V. cholerae* Table (1) [16].

The sensitivity, specificity, and diagnostic accuracy ,positive and negative predictive value (PPV, NPV) of the results were calculated by equations: d / c+d×100 = specificity a / a + b×100 = sensitivity a= Total of true positive, b= Total of false positive, c= Total of false negative and d= Total of true negative, Diagnostic accuracy = sensitivity +specificity/ 2 [19].

Results and Discussion

In the present work the identification of *V. cholerae* showed yellow colonies on TCBS agar purple colour on HiCrom agar and translucent non lactose fermenting pale colonies on MacConkey agar According to culture method showed 30 cultural samples is (13/30) (43.33%) of from bird's feces, and (10/30) (33.33%) from hand swabs samples are positive for *V. cholerae* while in biochemical tests; 10/ 30(33.33%) of bird's feces, and 3/30(10%) of hand swabs samples are positive for *V. cholerae*. On the other hand, in vitek2 diagnostic kit 2/30 (6.7%) birds feces, and 2/30(6.7%) hand swabs samples show positive results. This study shows that traditional methods are not always able to discriminate and identification these strains that isolated PCR technique shows only 7/30 (23.33%) birds feces, and 2/30(6.66%) hand swabs are positive. the PCR results of the sodB primer are effectively amplified at 248 bp primer (at 58ºC), Agarose 2%, and stained with red safe dye. The isolates are all positive for the sodb gene, which is typical for pathogenic *V. cholerae*. The results are observed under UV light as in Fig. 1.

Out of 30 culture sample, 23 were positive by culture method and 9 positives by PCR. So, the sensitivity, specificity, PPV, NPV and accuracy rates of PCR were (100%, 33%, 39%, 100% and 66.5%) respectively as demonstrated in (Table 2).

The sensitivity, specificity, PPV, NPV and accuracy rate of PCR were (100%, 81%, 69%, 100% and 90.5%) in results of biochemical tests compared to PCR(Table 3). Whereas, the sensitivity, specificity, PPV, NPV and accuracy rate of PCR were (44%, 100%, 100%,81% and 72%) in results of Vitek and PCR (Table 4).

In the present study, all of the conventional methods' findings matched with those of [20-28].. The culture method, and PCR results in this study revealed significant differences, between culture method and the PCR, PCR was is more sensitive than culture methods, the culture methods have less sensitivity and false positive results. According to the results of comparison between the PCR method and other tests in the current study, Conventional methods employed to identifying *V. cholerae*, are time-consuming and arduous, the molecular approach for detecting *V. cholerae* is faster, more accurate and specific high accuracy when employing *sodB* specific primer for specific detection due to a variety of bacteria that found in feces can grow and create yellow colonies on TCBS agar with false positive results in identification as described by [29, 30].In previous study found that PCR results showed high specificity compared to bacteria culture ,biochemical tests, API 20E respectively [31,32]. Vibrio can be an issue because of the variety in biochemical properties and the inability to completely isolate some *Vibrio spp*. For the rapid, sensitive, and accurate detection of small amounts of microorganisms, PCR is preferable to other molecular biology-based methods [33]. The present findings showed that the automated microbiological system used by Vitek 2 Compact, not always identify *V. cholerae* strains and this result agree with previous study of [34], In another study, 20 isolates of *V. cholerae* identify by PCR, while in Vitek 2 identified 10 isolates of *V. cholerae* and other 10 isolates were recognized as members of Aeromonadaceae, and Enterobacteriaceae [35]. Furthermore, other study showed PCR high specificity and sensitive in with low microbial concentrations in comparison with cultural, biochemical as mentioned by [17,33,36]. On the other hands, for the detection of *V.cholerae* numerous researcher employed PCR techniques all of them found that PCR methods were more rapid, accurate, and specific approaches for detection *V. cholerae*. [37-44].The ratio of *V. cholerae* identification by the *sodB* gene in the current investigation was 7/9 (77.77%) represented by the ratio from avian feces. Furthermore, prior researches by [45, 46], was performed on the *V. cholerae* according to the PCR results by using (sodB gene). Based on these findings, the isolation rate of *V. cholerae* in birds is a result of variations in diagnosis techniques, the precision of using gene primers, and flaws in DNA extraction [24]. The current study, PCR findings record 2/9 (22.22%) isolates of *V. cholerae in* human hand swabs, there is no study in Iraq for detection of *V. cholerae* from human hand swabs of owner birds, in other studies in the world reported other type of *Vibrio spp.* by molecular detection in samples from hand swabs of fish handlers [25]. In addition, *V.cholerae* recorded detection by PCR in farmer infected by contaminated open wounds [48] . Moreover, in another study also found *V. cholerae* in samples from hands of workers [49]. However, this study indicates *that V. cholerae*, can identified incorrect by conventional methods. The results should motivate diagnostic labs and microbiologists to perform conventional, PCR tests to identification to avoid accidentally missing *V. cholerae* [50]. This study found that isolation of *V.cholerae* in birds and owners is the first study in Iraq.

Conclusions

Application of PCR method should be considered as an important tools, i.e. a rapid and very sensitive, accurate and essential technique for the detection and screening (epidemiological investigations) of the public health threat, especially monitoring the strains of *V. cholerae*, to allow fully understanding its modes of transmission (sources and vehicles).

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Authors contributions

All Authors worked simultaneously to collect samples, data and statistically analyse it.

Conflict of interest

All authors declare that they have no conflicts of interest

TABLE 1. Primer used in the study

Primer	Sequence (5′→3′ direction)
	sodB* gene of *V. cholerae
F	**5'-AAGACCTCAACTGGCGGTA-3'**
R	**5'-GAAGTGTTAGTGATCGCCAGAGT-3'**

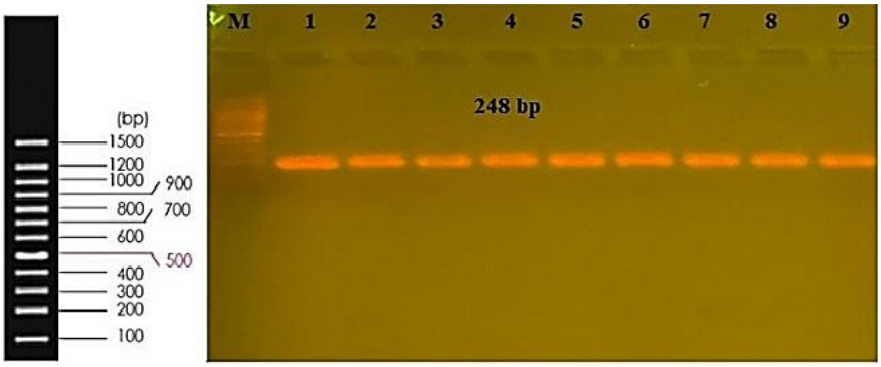

Fig. 1. Gel electrophoresis for PCR product of sodB primer show 248 bp Primer at 58 C, Agarose 2%, at 5 volts for 60min and Visualized under U.V light after staining with red safe stain.

TABLE 2. PCR test and culture method for diagnosis of *V. cholerae*

		PCR +ve	PCR -ve	Total
Culture method	+ve	**9**	**14**	**23**
Culture method	-ve	**0**	**7**	**7**
Total		**9**	**21**	**30**

TABLE 3. PCR test with biochemical tests for diagnosis of *V. cholerae*

Test		PCR (+ve)	PCR (-ve)	Total
Biochemical tests	+ve	**9**	**4**	**13**
Biochemical tests	_ve	**0**	**17**	**17**
Total		**9**	**21**	**30**

TABLE 4. PCR test with VITEK2 for diagnosis of *V. cholerae*

Test		PCR (+ve)	PCR(-ve)	Total
Vitek2	**+ve**	**4**	**0**	**4**
Vitek2	**-ve**	**5**	**21**	**26**
Total		**9**	**21**	**30**

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مقارنة بين التوصيف الروتيني والجزيئي لضمة الكوليرا المعزولة من الانسان والطيور

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[١] فرع الاحياء المجهرية - كلية الطب البيطري - جامعة بغداد - بغداد -العرا ق.
[٢] فرع الطب الباطني الوقا ئي - كلية الطب البيطري - جامعة بغداد - بغداد - العرا ق.

هدفت الدراسه إلى المقارنة بين التوصيف الروتيني والجزيئي في تقييم مدى انتشار ضمة الكوليرا والمقارنة بين طرق التشخيص، تم جمع ٩٠ عينة تشمل ٤٠ من براز الطيور من مزارع الطيور ومحلات تربية الطيور للبط والدجاج والحمام والطيور الأليفة و٥٠ مسحةمن ايادي أصحاب الطيور من(الذكور و الإناث) من أماكن مختلفة في مدينة الحرية، مدينة أبو غريب، منطقة اليرموك، منطقة الجامعة في محافظة بغداد، للمدة من شباط ٢٠٢١ الى شباط ٢٠٢٢، بهدف تقييم الإجراءات التشخيصية المختبرية الروتينية في تشخيص المرض. تم عزل ضمة الكوليرا ومقارنتها مع الطرق الجزيئية مثل تفاعل البلمرة المتسلسل (PCR). تم عزل وتعريف ضمة الكوليرا باستخدام طريقة الزراعة بالإضافة إلى الاختبارات الكيموحيوية ونظام فيتيك أظهرت النتائج أنه في طريقة الاستزراع نسبة العزل (٤٣,٣٣٪) من براز الطيور، و(٣٣,٣٣٪) في مسحات اليد، وفي الاختبارات الكيموحيوية (٣٣,٣٣٪) في براز الطيور، و(١٠٪) من مسحات اليد وفي نظام فيتيك (٦,٧٪) لكل من براز الطيور ومسحات اليد. استخدام الجين sodB لبروتين الغشاء الخارجي لضمة الكوليرا بناءً على نتائج تفاعل البوليميراز المتسلسل، كان معدل عزل ضمة الكوليرا من عينات براز الطيور ٢٣,٣٪. و٦,٦٪ من عينات مسحة اليد، أظهرت نتائج تفاعل البوليميراز المتسلسل أن هناك حساسية عالية بنسبة ١٠٠٪ بالمقارنة مع طرق الزرع والفحوص الكيميائية الحيوية، في حين بلغت٤٤ ٪ مع نظام فيتيك ٢ على التوالي. قد يكون نتيجة تحديد ضمة الكوليرا عن طريق التشخيص الروتيني غير صحيح ان استخدام تفاعل البوليميراز المتسلسل يشكل طريقةسريعة دقيقة ومحددة للتشخيص الصحيح

الكلمات المفتاحية : الكوليرا، نظام Vitek 2، sodBGene، أصحاب الطيور.