

Identification of *Vibrio Cholerae* using Analytical Profile Index (API 20E)

¹Christopher E. Ezeamagu, ¹Mande Garuba and ²Toyosi F. Osisami

¹Chemistry Department, Federal College of Education, Technical Gusau, Zamfara State.

²Department of Microbiology, Babcock University, Nigeria.

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ABSTRACT

Cholera outbreak have resulted in the deaths of millions of people globally. The cholera-causing *Vibrio cholerae*, has multiple serogroups, and produces epidemics when two strains, O1 and O139, are present. With the exception of recent, rare instances, *V. cholerae* O139 caused prior outbreaks; however, *V. cholerae* O1 was responsible for all following epidemics. Hence, identification of the *V. cholerae* is an important step in the control of cholera outbreak especially in the local community where inadequate facilities for prognosis limit prompt treatment of patients. A total of 15 isolates previously identified with molecular method were subjected to TCBS Agar and Analytical Profile Index. All the sucrose-fermenting colonies on TCBS agar were correctly identified with API kit. The study concluded that API kit can be substituted for molecular method in prognosis of Cholera outbreak especially in resource-limited Countries due cost implication.

Keyword: API, Cholera, TCBS, Water contamination, *Vibrio cholerae*.

INTRODUCTION

Cholera is believed to have its origins in the Ganges Delta in India and spread over the world through seven subsequent pandemics that resulted in the deaths of millions of people on each of the seven continents. The seventh pandemic, which affected a disproportionately significant amount of the world's population, originated in South Asia in 1961, traveled to Africa in 1971, and then reached the Americas in 1991. Within 40 years, the World Health Organization received 3,221,050 probable cholera cases from African countries, making up 46% of all cases recorded internationally (Mengel et al., 2014). About 99% of all fatalities and 86% of all cases that were reported around the world in 2011 were caused by the cholera pandemic. There are just two strains of the cholera-causing *Vibrio cholerae*, O1 and O139.

The cholera-causing *Vibrio cholerae*, which has multiple serogroups, only produces epidemics when two strains, O1 and O139, are present. With the exception of recent, rare instances, *V. cholerae* O139 caused prior outbreaks; however, *V. cholerae* O1 was responsible for all following epidemics (WHO, 2018). The outbreak often occurs year-round during the rainy season, and the availability of sanitary facilities and clean water is significantly connected with the spread of the disease. At-risk regions frequently include slums in peri-urban areas and camps for internally displaced people or refugees because they lack even the most basic necessities like clean water and sanitation (WHO, 2018).

Between January and June 22, 2018, a total of 13,009 suspected cases in twelve states of Nigeria were reported, with 116 fatalities. The age categories most afflicted were 1-4 years (29.2%) and 5-14 years

(24.8%), with a case fatality rate of 0.89% overall (NCDC, 2018). Zamfara State recorded the second-highest number of cholera cases in Nigeria in 2018 (729), along with 9 fatalities. This outbreak is typically infrequent and necessitates quick action. The O1 and O139 are two well-known *Vibrio cholerae* strains that have been linked to cholera outbreaks around the world. Although the epidemiology of cholera is shifting, a prior report on an outbreak in Nigeria revealed that it was caused by unusual El Tor strains with virulent traits and resistance determinants (Marin et al., 2013). Hence, identification of the *V. cholerae* is an important step in the control of cholera outbreak especially in the local community where inadequate facilities for prognosis limit prompt treatment of patients. The purpose of this study was to isolate and identify *V. cholerae* using both phenotypic (Thiosulfate Citrate Bile Salts Sucrose (TCBS) Agar analytical profile index (API).

Sample Collection and Isolation

Samples were taken from sampling locations and placed into sterile universal bottles in about 100 mL volume. The local government areas of Tsafe (3), Zurmi (2), Maradun (1), Talata Mafara (2), Gusau (3), Bungudu (13), Birnin Magaji/Kiyaw (1), and Shinkafi (12) provided. The samples were processed in a 24- to 48-hour period after being delivered to the laboratory in cold packs. The isolation of vibrio species was phenotypically determined using thiosulfate Citrate Bile Salts Sucrose (TCBS) Agar (Hopebio, China). To provide additional characterization, API 20E was employed following the manufacturer's instructions. Briefly, the manufacturer's instructions were followed for inoculating an API 20E strip (Biomerieux Industries, France) with a single colony from an overnight culture on nutritional agar with 2% NaCl, suspended in sterile saline water (0.85% NaCl) for each isolate. Then incubation was done within 16-24h at 37°C. After incubation, Utilizing the API 20E analytical profile index, a set of 21 reduced biochemical assays were analyzed.

RESULTS

A total of 15 isolates; well (1), rivers (11), streams (3), were obtained from 38 samples collected. The 15 sucrose-fermenting colonies on TCBS agar in collection. All were able to grow on NA and produced an oxidase-positive reaction. The API 20E technique allowed for the excellent identification of these presumed isolates as *V. cholerae* (Table 1).

Table 1: Phenotypic and biochemical identification of *V. cholerae*

Water type	Sample source	Sucrose-positive	Growth on NA	Growth on TCBS	API 20E profile ID
River	Bungudu	+	+	+	<i>V. cholerae</i>
River	Bungudu	+	+	+	<i>V. cholerae</i>
River	Bungudu	+	+	+	<i>V. cholerae</i>
River	Bungudu	+	+	+	<i>V. cholerae</i>
River	Bungudu	+	+	+	<i>V. cholerae</i>
River	Shinkafi	+	+	+	<i>V. cholerae</i>
River	Shinkafi	+	+	+	<i>V. cholerae</i>
River	Shinkafi	+	+	+	<i>V. cholerae</i>
River	Shinkafi	+	+	+	<i>V. cholerae</i>
River	Shinkafi	+	+	+	<i>V. cholerae</i>
River	Talata Mafara	+	+	+	<i>V. cholerae</i>
Stream	Tsafe	+	+	+	<i>V. cholerae</i>
Stream	Birnin Magaji/Kiyaw	+	+	+	<i>V. cholerae</i>

Stream	Zurmi	+	+	+	<i>V. cholerae</i>
Well	Gusau	+	+	+	<i>V. cholerae</i>

DISCUSSION

Cholera has been flagged, over the years, as a disease of public health importance due to many epidemics that have happened across the world and the lives that have been lost to the disease. In Nigeria, especially the northern states, Cholera has become a growing endemic and this study investigated the presence of the pathogen, *Vibrio cholerae*, in several water sources available for drinking to the people of Zamfara state. The results of this study show that there was significant occurrence of *Vibrio cholerae* in the samples collected, with the highest occurrence from the river sources. This agrees with reports from Asamoah (2023) and Abioye et al. (2021) showing that the largest percentage of *Vibrio cholerae* were found in the surface water.

The high prevalence of *Vibrio cholerae* in the rivers in a rural areas like these could be attributed to contamination in drinking water sources. Many areas in the state have water sources that are prone to faecal contamination, laundry activities and deposit of waste effluents while still being the source of drinking water. It has long been understood that surface water is primarily responsible for the transmission of cholera (Nwabor et al., 2016). The water from the well showed the least frequency of all the samples collected and this agrees with studies by Asamoah (2023).

This study shows that surface water is more implicated in the occurrence and spread of *Vibrio cholerae*. Because surface water frequently has larger concentrations of nutrients and organic materials, it creates a more ideal habitat for microbial growth (Cheng et al., 2022). However, surface water is also more vulnerable to alterations in the environment and human-caused contamination, which can result in the introduction of pathogens and other dangerous microbes (Smith & Casadevall, 2022; Bilal et al., 2023).

The difficulties Nigeria faces with regard to health and water resources were demonstrated by this study and other research on domestic water consumption in rural communities (Aikowe & Mazancová, 2021). Various reasons such as lack of proper infrastructures for waste disposal (Akhtar et al., 2021), increasing agricultural land use in rural areas (Ahmad et al., 2021) are contributing factors to the high level contamination in surface water.

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