African Journal of
Microbiology Research

ACADEMIC JOURNALS
expand your knowledge

About AJMR

The African Journal of Microbiology Research (AJMR) is a peer reviewed journal. The journal is published weekly and covers all areas of subject as Environmental Microbiology, Clinical Microbiology, Immunology, Virology, Bacteriology, Phycology, Molecular and Cellular Biology, Molecular Microbiology, Food Microbiology, Mycology and Parasitology, Microbial Ecology, Probiotics and Prebiotics and Industrial Microbiology.

Indexing

CAB Abstracts, CABI's Global Health Database, Chemical Abstracts (CAS Source Index) Dimensions Database, Google Scholar, Matrix of Information for The Analysis of Journals (MIAR), Microsoft Academic, Research Gate

Open Access Policy

Open Access is a publication model that enables the dissemination of research articles to the global community without restriction through the internet. All articles published under open access can be accessed by anyone with internet connection.

The African Journal of Microbiology Research is an Open Access journal. Abstracts and full texts of all articles published in this journal are freely accessible to everyone immediately after publication without any form of restriction.

Article License

Self-Archiving Policy

The African Journal of Microbiology Research is a RoMEO green journal. This permits authors to archive any version of their article they find most suitable, including the published version on their institutional repository and any other suitable website.
Please see http://www.sherpa.ac.uk/romeo/search.php?issn=1684-5315

Digital Archiving Policy

The African Journal of Microbiology Research is committed to the long-term preservation of its content. All articles published by the journal are preserved by Portico. In addition, the journal encourages authors to archive the published version of their articles on their institutional repositories and as well as other appropriate websites.
https://www.portico.org/publishers/ajournals/

Metadata Harvesting

The African Journal of Microbiology Research encourages metadata harvesting of all its content. The journal fully supports and implement the OAI version 2.0, which comes in a standard XML format. See Harvesting Parameter

Memberships and Standards

OPEN ACCESS

Academic Journals strongly supports the Open Access initiative. Abstracts and full texts of all articles published by Academic Journals are freely accessible to everyone immediately after publication.

creative commons

All articles published by Academic Journals are licensed under the Creative Commons Attribution 4.0 International License (CC BY 4.0). This permits anyone to copy, redistribute, remix, transmit and adapt the work provided the original work and source is appropriately cited.

Crossref

Crossref is an association of scholarly publishers that developed Digital Object Identification (DOI) system for the unique identification published materials. Academic Journals is a member of Crossref and uses the DOI system. All articles published by Academic Journals are issued DOI.

Similarity Check powered by iThenticate is an initiative started by CrossRef to help its members actively engage in efforts to prevent scholarly and professional plagiarism. Academic Journals is a member of Similarity Check.

CrossRef Cited-by Linking (formerly Forward Linking) is a service that allows you to discover how your publications are being cited and to incorporate that information into your online publication platform. Academic Journals is a member of CrossRef Cited-by.

<idpf>

Academic Journals is a member of the International Digital Publishing Forum (IDPF).
The IDPF is the global trade and standards organization dedicated to the development and promotion of electronic publishing and content consumption.

Contact

Editors

Editors

Table of Content

AJ ACADEMIC JOURNALS
expand your knowledge

African Journal of Microbiology Research

Full Length Research Paper

Assessment of bacteriological quality of water sources from an agrarian settlement in South-East Nigeria

Emmanuel Ikechukwu Nnamonu[1*], Felix Joel Ugwu[1], Obiageli Constance Ejilibe[1], Ogonna Christiana Ani[2], Paul Emenike Martins[3], Samuel Osemedua Onyeidu[4] and Blessing Uchechukwu Onyeidu[5]

[1]Department of Biology, Federal College of Education, Eha-Amufu, Enugu State, Nigeria.
[2]Department of Applied Biology, Ebonyi State University Abakaliki, Ebonyi State, Nigeria.
[3]Environmental Pollution Control, Faculty of Environmental and Life Sciences, University of Southampton, Highfield Campus, Southampton, Hampshire, SO17 1BJ, United Kingdom.
[4]Department of Information and Communication Technology, ICT University Yaounde, Cameroon.
[5]Department of Home Economics, Federal College of Education, Eha-Amufu, Enugu State, Nigeria.

This study assessed the bacteriological quality of groundwater, rainwater and surface water sourced from an agrarian settlement in South-eastern Nigeria. Agamede, Amede, Ihenyi, Mgbuji and Umuhu communities in Eha-Amufu served as sampling locations. Samples were sourced from River Ebenyi, hand-dug wells and rooftop harvested rainwater. Bacterial isolation, identification and enumeration followed standard methods. Coliform counts were above WHO permissible standard except groundwater sourced at Mgbuji. Whereas a significant difference occurred in the means of coliform counts of lactose-fermenters (LF) of groundwater and rainwater, no significant difference occurred among non-lactose fermenters (NLF) across all locations. There was no significant difference between the mean coliform colony count of LF and NLF across surface water in different locations. Bayesian Paired Samples T-Test and Post-Hoc showed no significant difference in the total colony count of LF and NLF coliforms and means of total colony count between and within water sources across stations. However, the mean total coliform count in rainwater was the highest. Our results demonstrate that water sources from the agrarian settlements were contaminated by coliforms whilst settlers and others that consume them via drinking and domestic purposes are vulnerable, especially the aged, children and immunocompromised.

Key words: Water quality, bacteriology, agrarian settlement, coliform.

INTRODUCTION

Comparatively, escalating demands for potable drinking water exist in urban than rural settlements because most people in rural settlements (especially agrarian settlements) consume poor quality and contaminated water out of ignorance of the huge public health implications. Quality water is cardinal to the survival,

livelihood and well-being of humans yet it is scare. Only 2.7% of the earth's water is freshwater (in lakes, rivers and ground) out of which only 1% is accessible (WHO, 2012). The inaccessible 1.7% is hidden in deep aquifers and glaciers. Consequently, humans suffer due do water scarcity. Water scarcity affects four out of every 10 people (WHO, 2012). This is exacerbated in regions with increased microbial contamination or pollution.

Beside cases of scarcity, anthropological activities such as: inadequate urban management, industrial pollution, over-fertilization, use of pesticides and agricultural wastewater (especially from animal husbandry) result in contamination of drinkable water sources to millions of people. Contaminated water transmits waterborne diseases such as cholera, diarrhoea, dysentery, hepatitis A, typhoid, and polio. By 2025, half of the world's population will be living in water-stressed areas (UNWWAP, 2014). It has been recorded that at least 2 billion people use drinking water source contaminated with faeces. Pathetically, majority of people without access to safe water are from Africa (UNWWAP, 2014; Martins et al., 2016; Nwabor et al., 2016). Contaminated drinking water is estimated to cause 485,000 annual diarrhoeal deaths, killing about 1.8 million children yearly and over 220 million people required preventative treatment for schistosomiasis in 2017 (WHO, 2012). In least developed countries, 22% of health care facilities have no water service. More so, approximately 3.1% of the global annual death (1.7 million) and 3.7% of the annual burden (disability) (54.2 million) are caused by the use of unsafe water (WHO, 2012).

In Nigeria, diarrhoea leads to over 70,000 annual deaths of children under five. More than 45 million people practice open defecation in Nigeria (FMWR, 2015) consequently contaminating natural water sources. Only 26.5% of the population use improved drinking water sources. Most people residing sideways of water bodies (especially in rural and agrarian settlements) still source and drink untreated water from lakes, rivers, springs, streams, wells and other water bodies regardless of the state of these water bodies with (FMWR, 2015; Martins et al., 2016; Nwabor et al., 2016). Although the practise of indiscriminate dumping and dispersal of wastes from home, hospitals, markets, factories and industries to water bodies is not recent, the continual adoption of this method of waste dispersal, especially during rainy season, will continue to be drawbacks in surface water backdrop. There is need for consistent monitoring of the rate and impact of microbial contamination of water bodies especially in rural and semi-urban areas where high dependence on surface and rain water is rife.

Objectives

This study was designed to assess the total colony count of different water sources from different communities in Eha-Amufu. Hence, the objectives include:

(1) Examining the distribution of total lactose-fermenting (LF) and non-lactose-fermenting (NLF) coliforms from different water sources and locations; to draw inference on the substrate and pollution source responsible for microbial presence and growth.
(2) To evaluate, within each water source, the variation of microbial load across different locations in Eha-Amufu.
(3) Compare total coliform count from different sources of water to communities in different locations in order to assess the source with higher health and environmental concern.

MATERIALS AND METHODS

Study area

Eha-Amufu is a town in South-eastern Nigeria. Coordinates – Universal Transverse Mercator (UTM): LN63; geographical coordinates in decimal degrees (WGS84): latitude: 6.667, longitude: 7.767; geographical coordinates in degrees minutes seconds (WGS84): latitude: 6 40' 00'', longitude: 7 46 ' 00''. Daylight: sunrise: 06:17:35; sunset: 18:08:08. It is located at latitude: 6° 39' 32.94'' N; longitude: 7°45 ' 34.60'' E. It is situated at elevation of 109 meters above sea level (http://www.tageo.com/index-e-ni-v-00-d-m2792190.htm). Eha-Amufu has an average temperature of 27.0°C and average precipitation of 1669 mm (CLIMATE-DATA.ORG). It is traversed by the Ebenyi River (ER) and its tributaries. The residents are dominantly of the Igbo speaking tribe and mainly rural farmers, fishermen, artisans and traders. Two distinct seasons occur in the area: the dry and wet seasons. The dry season stretches from November to April or May while the rainy season begins around May and ends in October (Nnamonu et al., 2018).

Sample collection

Surface water samples were sourced from three sampling points along each of these Ebenyi Rivers: Ebenyi Agamede, Ebenyi Amede, Ebenyi Ihenyi, Ebenyi Mgbuji and Ebenyi Umuhu using 200 ml sterilized borosilicate glass bottles. Ground water and rain water were as well collected from three randomly selected locations in each of these communities: Agamede, Amede, Ihenyi, Mgbuji and Umuhu. Ground water was sourced from hand-dug wells while rain water was harvested directly from rooftop at three randomly selected locations in each of these communities: Agamede, Amede, Ihenyi, Mgbuji and Umuhu. All samples were collected using 200 ml sterilized borosilicate glass bottles. All the 200 ml sterilized borosilicate glass bottles were immediately labelled and transported in ice-pack to the laboratory for bacteriological analysis within 6 h of sample collections.

Bacteriological analyses

Bacterial isolation, identification and enumeration

Isolation and identification of microorganisms were carried out with the following media: nutrient agar, Simon's citrate agar and urea agar base (manufactured by Hardy Diagnostics; 1430 West McCoy Lane, Santa Maria, CA 93455, USA); MacConkey agar, peptone water, kligler iron agar and urea medium (manufactured by Central Drug House Limited, NewDelhi, India).

In the laboratory, 10 µl of each water sample was inoculated onto a MacConkey agar plate, and evenly spread using sterile glass spreader. Each sample was duplicated. The MacConkey plates

were incubated at 37°C for 24 h. The plates were examined and isolates were enumerated based on grown colonial morphology, colour and texture. Different colonies were subcultured to obtain distinct colonies. Distinct colonies from the agar were examined for ability to ferment lactose. Non-lactose fermenting colonies were further inoculated onto deoxycholate citrate agar (DCA) and incubated overnight at 37°C. A representative of each distinct colony type was Gram stained and subjected to biochemical tests according to the WHO Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance in the Developing World (WHO/CDS/CSR/ RMD/2003.6).

Growth on Kligler iron agar

Presumptive identification was based on the reaction of the isolate on Kligler iron agar (KIA). Discrete colonies were carefully picked from the agar media using pointed inoculating wire loop. Tubes of KIA were inoculated by stabbing the butt and streaking the surface of the slant. The caps of the tubes were loosened before incubation. After incubation for 24 h at 37°C, the KIA slants were observed for reactions and results recorded. Yellowing of the butt indicated glucose fermentation; yellowing of slant indicated lactose fermentation; while reddening of the slant indicated inability of the organism to ferment lactose. Gas production was indicated by air bubbles, cracks or displacement of the medium. Hydrogen sulphide production was indicated by blackening of the medium

Sulfide-indole-motility (SIM) screening test

Sulfide-indole-motility medium (SIM) is a commercially available combination medium that combines three tests in a single tube: hydrogen sulfide (H_2S) production, indole production, and motility. The indole reaction is not useful for screening suspected *Shigella* isolates because strains vary in their reactions in this test. SIM medium was prepared from the dehydrated medium according to manufacturer's instruction. *Escherichia coli,* which is indole positive, H_2S negative and motility positive, was used as control. The SIM media were inoculated with a straight inoculating wire, by making a single stab about 1-2 cm down into the medium and was incubated overnight at 37°C. The surface of the motility agar was dry when used, since moisture is known to cause a non-motile organism to grow down the sides of the agar, creating a haze of growth and appearing to be motile. The motility reaction in SIM was indicated by the presence of diffused growth (appearing as clouding of the medium) away from the line of inoculation. The organisms that did not grow out from the line of inoculation were recorded as non-motile. As in Kligler iron agar, H_2S production was indicated by blackening of the medium. Indole production was tested by adding 0.5 ml of Kovac's reagent to the tube. Indole positive organisms produced red colour at the top of the test tube.

Viable cell count

Number of colonies was determined by converting the number of colonies per 10 µl to colony forming unit per 1000 µl (CFU/ml).

Site comparison and data analyses

Since we were mainly interested in the coliform count in different water sources across different locations within the study area, we compared the coliform counts using Bayesian statistical analysis due to its robustness which considers the dynamics and variations (prior probabilities) across sampling time and flow gradient (such as upstream to downstream) of water sources. One-way analysis of variance (ANOVA) was conducted using JASP software (version 0.9.2). Our hypotheses were that substrate type and water source have effect in coliform counts across different locations. Also, we hypothesised that a significant difference in coliform count exists within each water source across different locations. Post-Hoc Test was used to compare the difference between the mean of coliform loads within sample groups. Bayes Factor (BF_{10}) of the Bayesian statistic is similar to the *p*-value of classical frequentist. Results of the Bayesian analyses were appropriately interpreted (Kass and Raftery, 1995). BF_{10} value up to three (3) was considered significant and showed evidence supporting the alternative hypothesis.

RESULTS

Table 1 and Figure 1 display Bayesian ANOVA comparisons showing BF_{10} and Post-Hoc test of compared samples and subsamples. Bayesian ANOVA was conducted to test the effect of different locations, substrate and water sources on the occurrence of coliform count. Colony counts were stated as dependent variable while location, substrate type (LF and NLF) and water source were stated as fixed factors where relevant.

There was a significant difference in the means of coliform counts in groundwater (GW) and rainwater (RW). Within the ground water samples with lactose fermenters (Figure 1a), Agamede had mean count of 2.667×10^2 and standard deviation (SD) of 2.517 while Amede had mean count of 2.333×10^2 and SD of 0.577. Ihenyi had the highest mean count of 3.0×10^2; however the SD was 0.0 because the values were essentially constant across the sample size (N). Other locations (Mgbuji and Umuhu) have zero mean count and zero SD (Figure 1a). There was no significant difference between the mean coliform colony count of non-lactose fermenters across ground water in different locations (Figure 1b). Agamede had mean count of 28.333×10^2 and SD of 12.50 while Amede had mean count of 14.67×10^2 and SD of 12.86. Umuhu had mean count of 11.67 and SD of 10.12. Ihenyi had the lowest mean count of 4.67×10^2 and SD of 4.16. Mg had zero mean count and zero SD (Figure 1b).

Similar to the GW, there was a significant difference between the coliform colony count of lactose-fermenters (LF) in rainwater (RW) across different locations (Figure 1e). Umuhu had the highest mean count of 54×10^2 and SD 10.82 followed by Agamede with mean count of 45×10^2 and SD 10.44 whilst Amede, Ihenyi and Mgbuji had comparatively low mean count of 19.33, 11.67 and 9.67 respectively. The SD was 16.17, 6.81 and 4.04. The main effect of the difference lies within the Mgbuji and Umuhu locations as showed by the Post-Hoc test (Table 1). There was no significant difference between the mean coliform colony count of non-lactose fermenters across rainwater in different locations. Although a significant BF_{10} 12.29 was detected as an analysis of effect, no significant difference in colony count was observed across variations of location. Mgbuji had the highest mean colony count of 250.33×10^2 and SD 146.28. Mean

Table 1. Bayesian ANOVA comparisons showing BF$_{10}$ and Post-Hoc test of compared samples and subsamples.

Sample	Compared subsamples	Bayes factor (BF$_{10}$)	Post-Hoc test (BF$_{10. C}$)
GW_LF	Locations (Ag, Am[a], Ih, Mg[b], Um[b])	3.897	4.288
GW_NLF	Locations (Ag, Am, Ih, Mg, Um)	2.622	Very low
Locations	LF_C and NLF_C	1.418	Very low
RW_LF	Locations (Ag, Am, Ih, Mg[a], Um[b])	31.59	3.778
RW_NLF	Locations (Ag, Am, Ih, Mg, Um)	12.29	Very low
SW_LF	Locations (Ag, Am, Ih, Mg, Um)	0.28	Very low
SW_NLF	Locations (Ag, Am, Ih, Mg, Um)	1.99	Very low
Total C_LF	Locations (Ag, Am, Ih, Mg, Um)	0.352	Insignificant
Total C_NLF	Locations (Ag, Am, Ih, Mg, Um)	0.457	Insignificant
Water source	RW, SW; GW	0.993	
	RW; SW		0.458
	RW; GW		1.527
	SW; GW		0.302

BF$_{10. C}$, Posterior odds or Corrected Bayes Factor; BF$_{10}$ from the value of 3 and above shows a significant difference in relationship. GW, groundwater; RW, rainwater; LF, lactose-fermenter; NLF, non-lactose fermenter, C, colony count; Ag, Agamede; Am, Amede; Ihenyi, Ih; Mg, Mgbuji; Um, Umuhu. [a,b] Locations with different superscript differ significantly and those with no superscript do not differ significantly or cannot be compared.

colony counts for Amede, Ihenyi, Agamede, and Umuhu were 23, 11.67, 0.67 and 0.67 with respective SD of 18.36, 12.50, 0.58 and 1.16 (Figure 1f).

There was no significant difference between the mean coliform colony count of lactose and non-lactose fermenters across surface water in different locations (Table 1). There were low BF$_{10}$ and lack of significance in variations of locations. Umuhu and Ihenyi had high mean colony count of 20 and 18.89 while Amede, Agamede and Mgbuji had low mean count of 4.22, 3.67 and 1.67 (Figure 1g).

There was weak BF across all models. There was no significant difference in the colony count across compared locations. The means of the locations were 93.11, 32.11, 19.11, 6.0 and 5.56 for Amede, Agamede, Ihenyi, Mgbuji and Umuhu separately (Figure 1i). Bayesian Paired Samples T-Test was carried out to test if there is a significant difference in the total colony count of LF and NLF coliforms. With BF$_{10}$ of 1.418 (Table 1), there was no significant difference across the priors tested. There is evidence in support of the null hypothesis that colony count between LF and NLF was evenly distributed across the locations. LF_C had a mean count of 11.72 x 10^2 with 95% Credible Interval (CI) 6.154 to 17.29. Colony of Non-lactose fermenter (NLF_C) had a mean count of 32.53 x 10^2 with 95% CI 15.28 to 49.79 (Figure 2).

The credible interval (CI) is overlapping at some extent, making the analysis between lactose fermenting and non-lactose fermenting insignificantly different from one another although both CI look different. Colony count of non-lactose-fermenters (NLF_C) was higher than colony of Lactose-fermenters (LF_C). This may suggest that the study area has multiple alternative substrates sustaining the proliferation of varieties of coliforms from many sources across all tested locations (Figure 2). There was

no significant difference between the mean of total coliform colony count of lactose and non-lactose fermenters across all water sources in different locations (Table 1). Figure 1c had weak BF across all models. No significant difference in the colony count across compared locations. The means of the locations were 22.80, 14.27, 11.73, 6.87 and 2.93 for Umuhu, Ihenyi, Agamede, Amede and Mgbuji separately. Figure 1d had weak BF across all models. No significant difference in the colony count across compared locations. The means of the locations were 63.40, 53.67, 25.07, 14.73 and 5.80 for Amede, Mgbuji, Agamede, Ihenyi and Umuhu independently.

Bayesian Paired Samples T-Test and Post-Hoc comparison were used to compare the means of total colony count between and within water sources. There was no significant difference in the total coliform count of different sources of water to the communities in different locations. This statistically suggests that all water sources (GW, SW and RW) have similar microbial load. However, the mean total coliform count in RW was the highest while GW had the least coliform count (Figure 3).

DISCUSSION

The observed alarming level of ignorance with regards to the grave side effects of consuming contaminated water among people in rural settlements (especially agrarian settlements) motivated the present study. This study specifically evaluated the bacteriological quality of ground, rain and surface water sourced from an agrarian settlement.

The bacteriological analysis of ground water (hand-dug well) showed that lactose fermenter (*E. coli*) was isolated from water sourced from wells in three communities
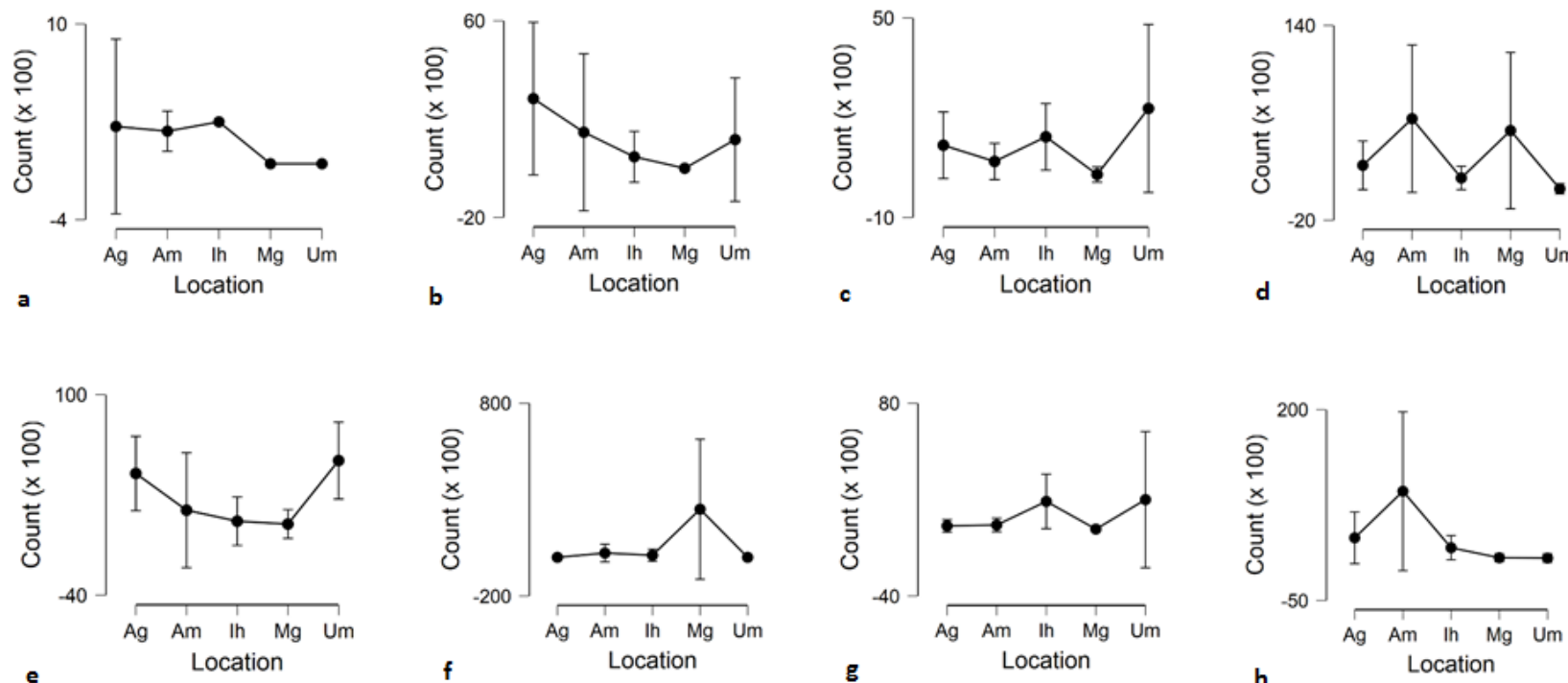
Figure 1. Comparison of lactose and non-lactose fermenting bacteria count in water samples (ground, rain and surface) sourced from Agamede, Amede, Ihenyi, Mgbuji and Umuhu in Eha-Amufu. **(a)** counts of lactose fermenters in groundwater **(b)** counts of non-lactose fermenters in groundwater **(c)** total colony count of lactose fermenters **(d)** total colony count of non-lactose fermenters **(e)** counts of lactose fermenters in rainwater **(f)** counts of non-lactose fermenters in rainwater **(g)** counts of lactose fermenters in surface water **(h)** counts of non-lactose fermenters in surface water. Ag, Agamede; Am, Amede; Ihenyi, Ih; Mg, Mgbuji; Um, Umuhu.

(Ihenyi, Agamede and Amede) while two communities (Mgbuji and Umuhu) recorded zero level of *E. coli*. These results consonant with many studies (Anyanwu and Okoli, 2012; Nwabor et al., 2016; Bolaji et al., 2017; Onuigbo et al., 2017; Obeta and Mamah, 2018). Specifically, the significant difference within this group was between the mean colony count of Amede and Mgbuji or Umuhu. This suggests that Mgbuji and Umuhu have lower level of faecal pollution compared to Amede. Contrary to the believe of some of the natives in the rural settlements that

nothing in water kills, *E. coli* and other feacal and total coliforms are infectious organisms of public health importance. The presence of these organisms indicates that there is animal or human waste contaminant in water. They cause intestinal infections, dysentery, hepatitis, typhoid fever, cholera, gastroenteritis which sometimes lead to other complications and death as well (Nwabor et al., 2016). Consequently, WHO has recommended that any water that contains these organisms should not be used for drinking, cooking nor processing of food, and similar domestic purposes

including brushing/ washing of mouth without disinfecting it first (WHO, 2011; Martins et al., 2016; Nwabor et al., 2016). We have observed in our study area that most hand-dug wells were not properly covered. When covers are available, they are not elevated, and this allows run-off to have its way into the wells during rainy season. Similarly, in some residence toilets and suck-away pits are not distanced / well positioned away from the wells (Nwabor et al., 2016). Pathetically, open filled / bush defecation is still practiced by some persons in the area.
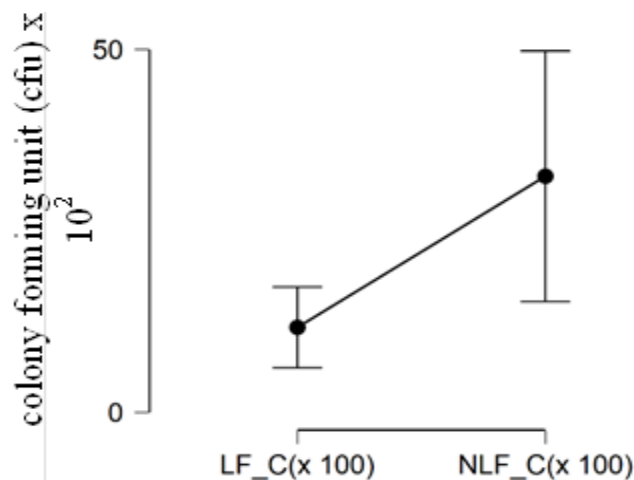
Figure 2. Comparative 95% credible interval plot of mean colony count between lactose fermenting (LF) and non-lactose-fermenting (NLF) coliforms, colony count (C) (x 10^2); Y-axis is colony forming unit (cfu) x 10^2 or Count (x 100).

Additionally, there is serious problem of poor sanitation. For instance, the kind / nature of buckets and other containers used in drawing out water from the hand-dug wells is not in any way a cause of concern for most persons in the area.

There was no significant difference between the mean coliform colony count of non-lactose fermenters across ground water in different locations. Whereas non-lactose fermenters count in ground water sourced from four communities (Agamede, Amede, Umuhu and Ihenyi) were above WHO permissible standard, ground water sourced from Mgbuji recorded zero count. This is in agreement with Anyanwu and Okoli (2012) and Obeta and Mamah (2018). We therefore tend to report that during the period of this study, ground water sourced from Mgbuji met the WHO bacteriological standard for drinking water. This may also imply that there is better sanitation and waste management practices at Mgbuji compared with other communities studied.

Similar to the groundwater, there was a significant difference between the coliform colony count of lactose-fermenters (LF) in rainwater (RW) across different locations (Figure 1e). The main effect of the difference lies within the Mgbuji and Umuhu locations as showed by the Post-Hoc test (Table 1). There was no significant difference between the mean coliform colony count of non-lactose fermenters across rainwater in different locations (Figure 1f). Although a significant BF_{10} 12.29 was detected as an analysis of effect, no significant difference in colony count was observed across variations of location. The coliform colony count of both lactose-fermenters and non-lactose fermenters at each location showed coliform counts were above WHO permissible standard. Residents in the study areas were observed to use this rainwater as drinking water without further treatment. They also used same water for food processing and cooking. Preparation of uncooked foods like 'Abacha', washing of fruits without addition of salt, rinsing of plates and pots, teeth brushing, and bathing were other activities involving the use of the contaminated rainwater.

The source of contamination of the rainwater could be as a result of poor hygienically way of harvesting the rain water, deposition of animal dungs on the rooftops (animals such as birds, squirrels, rodents and other small mammals). High settling rate of microbes on rooftops of some houses, lack of and derelict management of toilets in the area especially open field defecation may have led to rampant defecation within the environment which could be contributory. There is need for continual reorientation of rural settlers, especially farmers, on the need to treat rain water harvested from rooftops and other sources before drinking and utilisation. There was a significant difference in the means of coliform counts in groundwater and rainwater. Rainwater recorded more coliforms that groundwater. This could be due to the hygiene level applied while harvesting the rainwater and suggests that rainwater in the area has higher risk of waterborne diseases than groundwater. There was no significant difference between the mean coliform colony count of lactose and non-lactose fermenters across surface water in different locations. However, coliform counts at individual locations showed values above WHO permissible standard. These results agree with Kolawo et al. (2011), Ihuma et al. (2016), Martins et al. (2016) and Nwabor et al. (2016). The commonest sources of these organisms in water are animal and human wastes (EPA, 2002). Candidly, open defecation system is still common in rural settlements and this serves as the major means of surface water contamination. Some nomadic
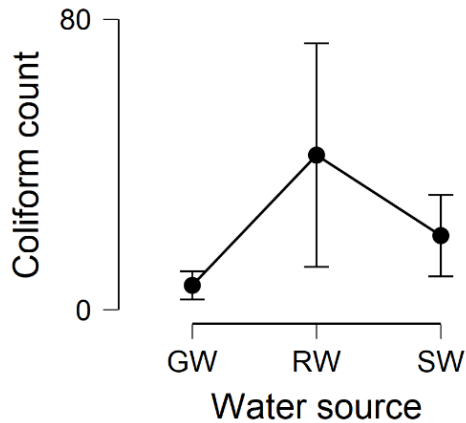
Figure 3. Comparison of mean coliform count of different water sources across all sampling sites. GW, groundwater; RW, rainwater; SW, surface water.

herdsmen often take their animals to available surface water for rehydration and these animals urinate, defecate, and transfer other bodily microbes into same water that rural settlers source water for drinking and other domestic purposes. Some abattoirs in rural settlements are sighted very close to rivers and streams for easy access to water. Rotten meats, animal dungs, blood and skeletal parts are often washed into the water bodies. These heavily contribute to microbial load of surface water. Open field waste disposal is also practiced in the studied rural settlements.

Bayesian Paired Samples T-Test was carried out to test if there is a significant difference in the total colony count of LF and NLF coliforms showed that there was no significant difference across the priors tested. This supports the null hypothesis that colony counts between LF and NLF were evenly distributed across the locations (Figure 2). This may suggest that the study area has multiple alternative substrates sustaining the proliferation of varieties of coliforms from many sources across all tested locations. This suggests that issues of hygiene and environmental sanitation be considered seriously in rural settlements.

Bayesian Paired Samples T-Test and Post-Hoc comparison showed no significant difference in the total coliform count of different sources of water to the communities in different locations suggesting that all water sources (GW, SW and RW) have similar microbial load. However, the mean total coliform count in RW was the highest while GW had the least coliform count (Figure 3). This depicts that rooftops and entire environment where the rain water is harvested are contaminated by coliforms. This also suggests high load of coliforms in the environment of the study area and a need for awareness and intervention on the locations with high dependence on RW and SW since considerably higher public health impact is more imminent than areas with available or high

dependence on GW (Figure 3).

Conclusions

This study has demonstrated that ground, rain and surface water sourced from rural agrarian settlements are vulnerable to coliform contamination. They also have poor bacteriological quality and are detrimental to health of those that drink and use them for other domestic purposes with regards to WHO recommendation. The state of this water should be of public health concern.

RECOMMENDATION

Further studies should adopt test methods describing sensitive and differential membrane filter (MF) medium; MI agar or MI broth should be used for the simultaneous detection and enumeration of both total coliforms (TC) and *E. coli* in water samples within 24 h or less based on their specific enzyme activities.

Limitations

The impact of meteorological variables like wind speed and direction in transferring coliforms from a source to location(s) was not studied. Also, the effect of environmental variables like humidity and temperature of the study site was not considered. The strain and pathogenicity of the isolates were not identified.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

REFERENCES

Anyanwu CU, Okoli EN (2012). Evaluation of the bacteriological and physicochemical quality of water supplies in Nsukka, Southeast, Nigeria. African Journal of Biotechnology 11:10868-10873.

Bolaji AS, Attah OT, Akanfe FA (2017). Bacteriological and physico-chemical analysis of underground water in Ede and its environs. International Conference of Science, Engineering and Environmental Technology 2:250-257.

Environment Protection Agency (EPA) (2002). US Environment Protection Agency, Safe Drinking Water Act Ammendment http://www.epa.gov/safe water/mcl. Html.

Federal Ministry of Water Resources (FMWR) (2015). Making Nigeria Open-Defecation Free by 2025. https://www.unicef.org/nigeria/media/1491/file/Nigeria-making-Nigeria-open-defecation-free-by-2025.pdf.pdf.

Ihuma JO, Adogo LY, Malgwi TD, Maikenti JI, Koggie AZ (2016). Bacteriological analysis of surface water obtained from Uke River in Karu, Nasarawa State, Nigeria. Asian Journal of Biology 1:1-6.

Kass RE, Raftery AE (1995). Bayes Factors. Journal of American Statistical Association 90:773-795.

Kolawo OM, Ajayi KT, Olayemi AB, Okoh AI (2011). Assessment of water quality in Asa River (Nigeria) and its indigenous *Clarias gariepinus* Fish. International Journal of Environmental Research and Public Health 8:4332-4352.

Martins PE, Nnamonu EI, Ejilibe OC, Uraih N (2016). Baseline agglutinin titre of Salmonella Enterica among healthy subjects in Idemili South, Anambra, Nigeria: An Aid in Medical Diagnosis. Journal of Medical and Applied Biosciences 8:16-30.

Nnamonu EI, Nkitnam EE, Ugwu FJ, Ejilibe OC, Ezenwosu SU, Ogbodo GU (2018). Physicochemical Assessment of Vulnerability of the River Ebenyi in Eha-Amufu and Environs, Southeast Nigeria. Annual Research and Review in Biology 27:1-9.

Nwabor OF, Nnamonu EI, Martins PE, Ani OC (2016). Water and Waterborne Diseases: A Review. International Journal of Tropical Disease and Health 12:1-14.

Obeta MC, Mamah KI (2018). Influence of environmental factors on the physico-chemical and bacteriological quality of well and borehole water in rural communities of Udenu Lga of Enugu State, Nigeria. Pakistan Journal of Nutrition 17:596-608.

Onuigbo AC, Onyia CE, Nwosu IG, Oyeagu U (2017). Impacts of bacterial pollution on hand-dug well water quality in Enugu, Enugu State, Nigeria. African Journal of Environmental Science and Technology 11:331-338.

United Nations World Water Assessment Programme (UNWWAP) (2014). The United Nations World Water Development Report: Water and Jobs. Paris: UNESCO.

World Health Organization (WHO) (2012). UN-Water Global Annual Assessment of Sanitation and Drinking Water Report: The Changes of Extending Sustaining Services. UN Water Report 2012 Switzerland. https://www.un.org/waterforlifedecade/pdf/glaas_report_2012_eng.pdf

World Health Organization (WHO) (2011). Guidelines for Drinking Water Quality, Genève: WHO Press, 4th ed. https://apps.who.int/iris/bitstream/handle/10665/44584/9789241548151_eng.pdf;jsessionid=651030D93F257A068767CB6BD65DC6C9?sequence=1

World Health Organization (WHO) (2003). Manual for the laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health importance in the developing world: Haemophilus influenzae, Neisseria meningitidis, Streptococcus pneumoniae, Neisseria gonorrhoea, Salmonella serotype Typhi, Shigella, and Vibrio cholera. https://apps.who.int/iris/bitstream/handle/10665/68554/WHO_CDS_CSR_RMD_2003.6.pdf?sequence=1&isAllowed=y

AJ ACADEMIC JOURNALS
expand your knowledge

African Journal of Microbiology Research

Full Length Research Paper

Evaluation of lactic acid bacteria isolated from fermented mustards in lowering hypercholesterol by using bile salt hydrolase (BSH) activity and bile salt de-conjugation

Shu Chen Wang[1], Chen Kai Chang[1], Shu Chia Chang[2], Chih Kuang Chiu[1] and Pin-Der Duh[1]*

[1]Department of Food Science and Technology, Chia Nan University of Pharmacy and Science, 60 Erren Road, Section 1, Rende District, Tainan, Taiwan R.O.C.
[2]Department of Biotechnology, Chia Nan University of Pharmacy and Science, 60 Erren Road, Section 1, Rende District, Tainan, Taiwan R.O.C.

This work evaluates lactic acid bacteria (LAB) obtained from fermented mustards for lowering cholesterol. The LAB strains' ability to reduce cholesterol *in vitro* was determined using activity of bile salt hydrolase (BSH) and deconjugation of bile salt *in vitro*. The LAB strains were examined for their ability to resist acid and bile salt. Strains that can reduce cholesterol adhere to Caco-2 cells and they were identified. 9 LAB isolates had higher total BSH activity (1.07 to 1.92 U/mL) than the reference strains. BSH specific activity range from 1.33 to 3.13 U/mg. Due to different protein contents in the cell extracts, there was no good correlation between the exact BSH activity and total BSH activity by most of the isolates. All of the isolates deconjugated sodium glycocholate, freeing cholic acid, from 1.02 to 2.64 mM. The tested isolates deconjugated sodium glycocholate at different levels. All tested strains exhibited acid tolerance and bile tolerance; they can survive well at pH 3.0 for 3 h and bile tolerance for 24 h. Adhesion of the tested strains to the Caco-2 cell lines, strain B0007 and B0008 strongly adhere in comparison to the control strains and other isolated strains. These results demonstrated positive effect of isolates on BSH activity, deconjugation and thereby leading to the probiotic potential of lowering cholesterol.

Key words: Lactic acid bacteria, acid- and bile-tolerant, bile salt hydrolase activity, deconjugation, adhesion activity.

INTRODUCTION

Hypercholesterol is a risk factor that causes cardiovascular disease. It is the number one cause of death in a lot of nations (Guo et al., 2019). The results from several epidemiological and clinical studies indicate a positive correlation between elevated total serum cholesterol levels and the occurrence of coronary heart

*Corresponding author. E-mail: ipdduh@mail.cnu.edu.tw. Tel: 88662664911. Fax: 88663662415.

disease (Pereira and Gibson, 2002). Increase in cholesterol levels causes the functional injury to the endothelium before morphological lesions develop (Sridevi et al., 2009). Cholesterol is a key substance causing coronary artery disease. The primary strategy for lowering cholesterol plasma levels and hindering the deposition of plaque inside the artery wall was studied (Raza et al., 2019). Drug therapy for hypercholesterolemic includes fibrate, statins and bile acid sequestrants, however the undesirable side effects of these compounds have caused concerns about their therapeutic use (Ward et al., 2019). Hence, there is requirement of a more natural method to decrease serum cholesterol concentration in humans. Milk fermented with lactobacilli or bifidobacteria was first demonstrated to exhibit the hypocholesterolemic effects in humans (Liong and Shal, 2005a).

The reduction of serum cholesterol could be an important health benefit of lactic acid bacteria (LAB). Various studies have shown that some lactobacilli could lower total cholesterol and low-density lipoprotein (LDL) cholesterol (Lang and Binggeli, 2002; Zhang et al., 2008). The exact mechanisms of serum cholesterol reduction by probiotic bacteria are not completely clear. Different hypothesis, such as assimilation (Wang et al., 2014; Reis et al., 2017), enzymatic hydrolysis and cholesterol co-precipitation properties (Lye et al., 2010) have been advanced to explain the cholesterol removal effect. Bile salt is one of the major routes of eliminating cholesterol from the body (Zhang et al., 2008). The bile salts are deconjugated in the small intestine, the solubility and emulsifying capacity decrease. Thus, the excretion of the free bile acids can reduce serum cholesterol level by increasing the formation of new bile salts from cholesterol (Liong and Shal, 2005b). Deconjugation has been included as one of the main activities of intestinal microorganisms that increase the synthesis of bile salts from serum cholesterol and thus reducing its uptake from the gut (Nguyen et al., 2007). BSH activity was detected in several bacterial species of the gastrointestinal tract, such as *Lactobacillus*, *Bifidobacterium*, *Clostridium*, *Enterococococcus* and *Bacteroides* spp (Shehata et al., 2016). *Lactobacilli*, Bifidobacteria are probiotic strains and were found to excrete bile salt hydrolase (BSH) (Liong and Shal, 2005b; Zhang et al., 2008), which catalyzes the hydrolysis of glycine or taurine-conjugated bile salts into amino acid residues and free bile salts. *L. plantarum* PH04 from infant feces was evaluated as a potential probiotic with cholesterol-lowering effect (Nguyen et al., 2007). *Bifidobacterium infantis* 17930 showed a high deconjugation ability to 3.22 mM and *B. longum* 536 showed high BSH activity of 1.37 U/ml (Liong and Shal, 2005b). Liong and Shah (2005a) found *L. acidophilus* ATCC33200, 4356 and 4962 and *L. casei* ASCC1521 showed higher deconjugation ability and BSH activity. Five *L. plantarum* and seven *L. pentosus* strains isolated from fermented olive, exhibited partial bile salt

hydrolase activity (Argyri et al., 2013). Five strains of lactic acid bacteria with BSH activity were preliminarily selected from swine origins (Guo et al., 2010). Probiotics are live microorganisms that improved its intestinal microbial balance (Vivek et al., 2019). Probiotics are normal components of the intestinal microflora in both humans and animals (Arepally and Goswami, 2019). They must have the ability to survive passage through the stomach and small intestine. Therefore, resistance to the low pH of the gastric juice and the bile salt in the small intestine and to prevent the attachment of pathogens and stimulate their removal from the infected intestinal tract is one of the important selection criteria for probiotic (Shehata et al., 2016; Delcarlo et al., 2019).

Green mustard is used to make fermented mustard (picked mustard green). It produced by fermentation through a mixed microbial population comprising LAB (Wang et al., 2014). This work aimed to examine the isolated lactic acid bacteria (LAB) from fermented mustard, and to show the ability of the screened Lactobacillus isolates to reduce cholesterol with BSH activity and to deconjugate bile salt. The isolates were used to test for acid tolerance, bile salt tolerance and adherence assay with Caco-2 cells.

MATERIALS AND METHODS

Bactria strains

Microbial colonies were got from fermented mustard and considered as LAB (Wang et al., 2014). Liquid fermented mustards were obtained from central and southern Taiwan farms. The liquid samples diluted were placed on the De Man, Rogosa and Sharpe (MRS) agar surface composed of 5 g/L calcium carbonate. This was followed by their incubation for 1-2 days at 37°C. There was a random selection of colonies of clear zones on MRS agar plates, followed by their purification. Gram-positive and catalase-negative strains were considered to be speculative LAB; they were kept in MRS agar plate at 4°C. BCRC 17010, BCRC 17474, BCRC 910236 are collected from Bioresource Collection and Research Center (BCRC), Hsinchu, Taiwan. All isolated strains were stored at -80℃. Prior to experimental use, BCRC 17010, BCRC 17474, BCRC 910236 obtained as reference strains, all strains were subcultured three times in MRS broth.

Bile salt hydrolase (BSH) assay activity

Sridevi et al. (2009)'s ninhydrin method was used for the BSH assay. The culture was grown shortly for 72 h at 37°C in MRS media and centrifuged at 12,000 *g* and 4°C for 2 min. Potassium phosphate buffer (pH 6.5) was used to wash the cell pellet, followed by centrifugation. Next was the determination of the pellets' wet weight. Cell pellet was suspended in 100 µL reaction mixture having 10 mM potassium phosphate buffer pH 6.5, 10 mM dithiothreitol (DTT), and 10 mM sodium glycodeoxycholate. The mixture was incubated at 37°C for 30 min; the reaction ended with the addition of 100 µL of trichloroacetic acid (15% w/v). The mixture was centrifuged and 50 µL of the supernatant was mixed with 50 µL of 2% ninhydrin reagent. Then, it was well mixed and heated for 14 min. The absorbance of the cooled sample was recorded at 570 nm. One unit of bile salt hydrolase activity was taken as the amount

of enzyme that freed 1 mole of amino acid from the substrate every minute. The benchmark curve was made using glycine. The same method above was used to determine the specific BSH activity for cell free extract. Lowry's technique (Liong and Shal, 2005a) was used to determine the concentrations of proteins in cell free extract. All experiments were duplicated.

Deconjugation of sodium glycocholate

Bile salt deconjugation was related to the deconjugated bile released and Liong and Shah (2005b)'s modified method was utilized for measuring the amount of free cholic acid by each culture released. 10 mL of culture after being incubated was modified to pH 7.0 with NaOH (1 N). Cells were centrifuged at 10,000 g (KUBOTA, KM-15200, Japan) and 4°C for 10 min. The supernatant was modified to pH1.0 with HCl (10 N). One milliliter of the supernatant was added in 2 mL of ethyl acetate and the mixture was vortexed for 1 min. Two milliliter of the ethyl acetate layer was moved to a glass tube and evaporated under nitrogen at 60°C. The residue was instantly melted in 1 mL of NaOH (0.01 N). After thorough mixing, 1 mL of furfuraldehyde (1%) and 1 mL of H_2SO_4 (16 N) were added, and the mixture was vortexed for 1 min before heating at 65°C in a water bath for 10 min. After cooling, 2 mL of glacialacetic acid was added and the mixture was vortexed for 1 min. The absorbance was read at 660 nm. The amount of cholic acid released was determined using cholic acid standard (Sigma Chemical Co., St. Louis, MO, USA). All experiments were duplicated.

Acid tolerance

The cultures acid tolerance was examined by incubation of the organisms in MRS broth with 0.30% oxigall supplement. The pH was modified in 3.0 and 2.0 with HCl and cultures were incubated at 37°C for 3 h. All the isolates were subcultured three times prior to the experiment; next they were centrifuged after the last subculture, and inoculated (1% v/v) into the broth. Plate count technique was used to monitor their growth (Liong and Shal, 2005c). The experiments were duplicated.

Bile tolerance

The resistance of LAB isolates to bile salt was evaluated. The MRS broths (0, 0.5, and 1.0% (w/v) of oxigall) were made, placed in 10 mL volumes and purified by heating 121°C for 15 min. Each of the LAB isolate was subcultured 3 times prior to the use of the experiment; next they were centrifuged after the last subculture, and inoculated (1% v/v) into the broth. The reaction mixture and MRS broth were incubated at 37°C for 24 h. Their growth was monitored with the plate count technique (Liong and Shal, 2005c). All the experiments were duplicated.

Adherence assay

The Caco-2cell-lines derived from a colon carcinoma were bought from the Bioresources Collection and Research Center (BCRC), Hsin-Chu, Taiwan. They grew normally in Dulbeco's modified Eagle's minimal essential medium (DMEM; GIBCOBRL Laboratories, NY, USA) having 1.0 mmol/L sodium pyruvate and 10% (v/v) fetal bovine serum and 50 unit/mL Penicillin-Streptomycin (GIBCO) supplements. For the assay, monolayers of Caco-2 cells were made on glass cover slips fixed in six-well tissue-culture plates (NUNC products, supplied by the Life Technologies, Auckland, New Zealand). The cells and all experiments were maintained with cell-lines at 37°C in 5% CO_2 atmosphere. Before

the test, PBS (phosphate buffered saline) was used to wash all LAB twice and followed by centrifugation at 2100 r/min for 5 min. Bacterial cells were suspended again in 1 mL DMEM. One hundred microliters of the suspension (1×10^8 CFU/mL) was moved to a washed monolayer of cells, and incubated for 2 h at 37°C in 5% CO_2. PBS buffer (pH 7.4) was used to wash the monolayers four times; they were placed in methanol, Gram stained (Baxter Scientific Products, McGraw Park, Miami, FL, USA) and microscope was used to examine them under an oil immersion lens. Gopal et al. (2001)'s method was used to count the numbers of LAB cells adhering to the cultured cell lines.

Identification of strains

API 50 CHL fermentation assays (BioMerieux, S.A., Marcy I'Etoile, France) and 16S rDNA sequence analysis was used to identify LAB isolates that are tolerance to acid and bile salt and with lowering cholesterol through physiological tests. Those mainly verified via API 50 CHL fermentation assays were based on the instruction method. In 16S rDNA sequence analysis, the PCR primers were designed from the 16S rDNA genes primers 27F/1492F (Tanner et al., 2000). For the PCR assay, the method of Michael et al. (2000) was followed. DNA purification kit (Promega, Madision, WI, USA) was used to purify the amplification products and Nucleic acid Synthesis and Analysis Core Laboratory (Cheng Kung University College of Medicine, Tainan, Taiwan) was used to sequence them. Sequence homologies were analyzed by making a comparison of the sequence got with those in the database of the DNA (http//www.ncbi.nim.nih.gov/BLAST).

Statistical analysis

All data were calculated as means (means ± SD). Statistical Analysis System software package was used for the Statistical analysis. ANOVA methods were used for the analysis of variance. Duncan's multiple range tests at a level of $P < 0.05$ were used to determine the significant differences between means.

RESULTS

Table 1 shows the bile salt hydrolase (BSH) activity of LAB isolates. All the isolates have different levels of BSH effects on sodium glycocholate. 9 isolates had higher total activity of BSH (1.07 to 1.92 U/mL) in comparison to reference strains, BCRC 10747, BCRC 17010 and BCRC 910236; BCRC 17010 and BCRC 910236 had the lowest total activity of BSH than the 9 isolated strains. BSH specific activity from 1.33 to 3.13 U/mg (Table 1). There was no good correlation between the precise BSH activity with total BSH activity in most of the isolates. B0106, B0024 and reference strains with the lowest total activity had lowest exact activity.

Deconjugation of LAB isolates

Sodium glycocholate was deconjugated by all the isolates freeing the cholic acid, from 1.02-2.64 mM (Table 2). In general, BCRC17010, BCRC 17474, B0007, B0011, B0106, B0113 showed a better deconjugation ability

Table 1. Bile salt hydroxylase activity of lactic acid bacteria.

Strain	Total activity (U /mL)	Total protein (mg/ mL)	Specific activity (U /mg protein)
BCRC 17010	0.56 ± 0.00^e	0.60 ± 0.01	0.93 ± 0.01^b
BCRC 17474	1.02 ± 0.02^b	0.92 ± 0.04	1.11 ± 0.02^b
BCRC910236	0.19 ± 0.06^d	0.47 ± 0.05	0.38 ± 0.03^c
B0007	1.71 ± 0.08^c	0.60 ± 0.02	2.84 ± 0.08^a
B0008	1.35 ± 0.29^a	0.61 ± 0.01	2.22 ± 0.08^f
B0010	1.37 ± 0.01^a	0.47 ± 0.015	2.91 ± 0.06^a
B0011	1.65 ± 0.08^c	0.53 ± 0.00	3.13 ± 0.18^h
B0024	1.23 ± 0.02^{ab}	0.92 ± 0.02	1.33 ± 0.04^d
B0032	1.92 ± 0.07^f	0.52 ± 0.00	3.73 ± 0.04^i
B0077	1.36 ± 0.05^a	0.46 ± 0.01	2.96 ± 0.03^a
B0106	1.07 ± 0.12^b	0.67 ± 0.04	1.62 ± 0.18^e
B0113	1.28 ± 0.05^a	0.52 ± 0.00	2.46 ± 0.04^g

*Values are means ± SD for seven rats per group. Values in the same column with different superscript letter [a,b] are significantly different at $p < 0.05$ analyzed by Duncan's test.

Table 2. Deconjugation of sodium glycodeoxycholate by lactic acid bacteria.

Strain	Cholic acid released (mM)
BCRC17010	2.35 ± 0.06^a
BCRC17474	2.64 ± 0.0^h
BCRC910236	1.02 ± 0.16^b
B0007	2.51 ± 0.06^g
B0008	1.54 ± 0.09^e
B0010	1.37 ± 0.03^c
B0011	2.23 ± 0.03^a
B0024	1.02 ± 0.02^b
B0032	1.35 ± 0.05^{cd}
B0077	1.46 ± 0.10^{de}
B0106	2.31 ± 0.05^a
B0113	1.97 ± 0.11^f

*Values are means ± SD for seven rats per group. Values in the same column with different superscript letter [a,b] are significantly different at $p < 0.05$ analyzed by Duncan's test.

which liberated more than 1.90 mM of cholic acid, while BCRC 910236 and B0024 deconjugated very poorly, releasing just 1.02 mM.

Acid tolerance

In this work, all the LAB isolates could withstand the simulated gastric juice at pH3 after incubating for 3 h (Table 3). These findings suggest that all tested strains tolerated acid at pH 3.0.

Bile salt tolerance

The impacts of diverse amounts of bile salt on the capability of the isolates are shown in Table 4. Lactobacilli grown in MRS broth lacking bile salt were the control. All the strains grew well in MRS broth lacking bile salt, but they had different levels of tolerance to bile from 10^7-10^8 CFU/mL after being incubated at 0.5% and 1% oxigall for 24 h. Among the tested LAB isolates B0024 had the highest tolerance to bile salt than other isolates.

Table 3. Effects of pH3.0 solution on viability of lactic acid bacteria

Strain	MRS broth (og CFU/ml)	Treatment with pH3.0 for 3 h (og CFU/ml)
BCRC17010	8.21±0.21	7.76±0.05
BCRC17474	9.64±0.26	7.56±0.03
BCRC910236	9.40±0.02	6.36±0.02
B0007*	9.73±0.08	8.80±0.02
B0008	9.50±0.06	8.76±0.01
B0010	9.32±0.05	8.75±0.04
B0011	9.89±0.04	8.55±0.03
B0024	9.45±0.05	8.77±0.01
B0032	9.62±0.14	9.72±0.02
B0077	9.54±0.12	9.54±0.01
B0106	9.52±0.20	9.46±0.01
B0113	9.20±0.06	9.22±0.09

*The viability of B0007 is from Wang et al. (2014).

Table 4. Effects of different concentration of bile salt on the viability of lactic acid bacteria.

Strain	MRS broth (log CFU/ml)	0.5% Bile-MRS (log CFU/ml)	1.0% Bile-MRS (log CFU/ml)
BCRC17010	8.21±0.21	8.27±0.02	8.16±0.12
BCRC17474	9.64±0.26	8.24±0.04	8.24±0.24
BCRC910236	9.40±0.02	8.36±0.01	8.43±0.13
B0007*	9.73±0.08	8.07±0.01	7.90±0.10
B0008	9.593±0.01	8.07±0.08	7.79±0.07
B0010	9.68±0.07	7.63±0.03	7.91±0.03
B0011	9.89±0.04	7.90±0.02	7.73±0.03
B0024	9.86±0.03	8.70±0.04	8.40±0.01
B0032	9.62±0.14	7.93±0.06	7.58±0.06
B0077	9.54±0.12	8.11±0.05	7.40±0.03
B0106	9.52±0.20	7.53±0.05	7.60±0.11
B0113	9.20±0.06	7.53±0.04	7.48±0.09

*The viability of B0007 is from Wang et al. (2014).

Strain identification

The physiological and biochemical characteristics using API 50 CHL for Lactobacilli identified all tested strains as LAB species. The sequences of the selected strains tallied with the 16S r-DNA sequences from the Gen-Bank database (website) and used to identify the LAB strains. According to the experimental results, B0010 was identified as *Lactobacillus brevis*, other isolates were identified as *Lactobacillus plantarum* using 16S rDNA analysis (Table 5). The data got to show that 16S rDNA sequence of these strains matched well with that of 16S rDNA nucleotide sequence of these strains were 99 to 100% similarity.

Adherence ability

Table 6 shows the adherence ability of the test strains on the Caco-2 cell lines. B0007 and B0008 revealed powerful ability to adhere unlike the control strains and other isolated strains. However, B0010, B0024, B0077, B0106 and B0113 revealed the inability of the system to adhere.

DISCUSSION

Different works have revealed that some lactobacilli can reduce total plasma cholesterol and low-density lipoprotein (LDL) cholesterol (Guo et al., 2019). Our previous studies showed that these isolates can eliminate cholesterol *in vitro* through the mechanisms of assimilation (Wang et al., 2014), and we want to investigate the deconjugation features of bile salt and these isolates' BSH activities. Of the 50 isolates from fermented mustard, 9 isolates displayed over 1.0 U/ml

Table 5. Identification of LAB isolates based on 16S rDNA sequence similarity

Isolate of LAB	Strain	GeneBank acc. no.	Sequence similarity (%)
B0008	*Lactobacillus plantarum*	EU807752	99
B0010	*Lactobacillus brevis*	CP000416	99
B0011	*Lactobacillus plantarum*	HM130542	99
B0024	*Lactobacillus plantarum*	EU419597	99
B0032	*Lactobacillus plantarum*	HM462423	100
B0077	*Lactobacillus plantarum*	GU125597	99
B0106	*Lactobacillus plantarum*	HM058789	99
B0113	*Lactobacillus plantarum*	FJ763580	99

Table 6. Adhesion of LAB strains to Caco-2 cell-line at normal state

Strain	Adhesion index[a]
BCRC17010[b]	51±7
BCRC17474[c]	17±11
BCRC910236[d]	81±17
B0007[b]	223±21
B0008	211±34
B0010	N.D.
B0011	4±3
B0024	N.D.
B0032	68±45
B0077	N.D.
B0106	N.D.
B0113	N.D.

[a]Expressed as the average number of LAB adhering to human intestine cells, counts is carried out on 20 randomized microscopic fields. [b]The adhesion index of B0007 is from Wang et al. (2014).

BSH activity. Guo et al. (2010) showed that of the 14 *Lactobacillus* strains isolated from the samples of swine origins, only 2 (14%) showed BSH activity; out of the 16 *Lactobacillus* strains isolated from the samples of koumiss, 7 (44%) had BSH activity. Several studies showed that elimination of cholesterol *in vitro* is connected to the activity of bile salt hydrolase (Shehata et al., 2016; Guo et al., 2019). Seven isolates reduced over 20% cholesterol and showed activity of bile salt hydrolase (BSH) (Shehata et al., 2016). Guo et al. (2019) used 5 fifferent bile salts to evaluate the *Lactobacillus plantrum* KLDS 1.0344 of BSH activity, the BSH activity of the strain KLDS was 1.04 U/mg towards sodium taurodeoxycholate, and 0.47 U/mg toward sodium glycodeoxycholate. Six isolated probiotics bacteria from human origin are involved in the production of bile salt hydrolase (BSH) that assists in the reduction of serum cholesterol (Miremadi et al., 2014). The enzyme, bile salt hydrolase is involved in the catalysis of bile salt deconjugation for the liberation of primary bile acids (Shehata et al., 2016). The highest total BSH activity

(1.72 U/ml) and specificity activity (3.73 U/mg) was displayed by B0032; some isolates had lower total BSH activity, but had higher specificity activity unlike the rest LAB strains (Table 1). There was no good correlation between exact BSH activity and total BSH activity by most of the strains of LAB because the cell extracts had different protein contents. Liong and Shah (2005a) and Guo et al. (2019) had the same results for lactobacillus isolates on varying bile salts.

Two strains of *Bifidobacteria* strains could deconjugate sodium glycocholate to lower serum cholesterol levels (Liong and Shal, 2005b). It is shown that all the strains were able to deconjugate sodium glycocholate bile salt very well at different levels. These results are like others (Miremadi et al., 2014; Ramasamy et al., 2010), in which *Lactobacillus* strains had higher deconjugating activity on the conjugates of sodium glycocholate. In this study, all tested isolates deconjugated sodium glycocholate bile salt varying degrees. Similar results were reported by 12 *Lactobacillus* isolates that deconjugated bile salts to different levels (Ramasamy et al., 2010).

pH 3 is normally used for gastric juice while pH 2 for 3 h is utilized as severe situation for the simulation of the stomach conditions (Tsai et al., 2005). *L. fermentum* strain 4-17 was capable of surviving at various conditions such as low pH values, bile salts exposure (Falah et al., 2019). The results showed that pH 2.0 obviously inhibited the strains. It was accepted in general that the strains with full tolerance to pH 3 for 3 h had relatively higher resistance to the low acidity. B0032, B0077, B0106 and B0113 displayed no difference viability before and after pH 3.0 for 3 h. The results show the tolerance to acid was strain-specific, like other studies (Tsai et al., 2005; Mishra and Prasad, 2005). The useful physiology of of human bile concentrations is from 0.3 to 0.5% (Dunne et al., 2001). In order to test the bile salt tolerance, 0.5 and 1.0% bile salt were used in this study. All the tested strains exhibited bile tolerance of 0.5 and 1.0% bile salt with varying degrees. The results suggest the tolerance to bile salt was similar to the report of Falah et al. (2019).

Researchers have given the suggestion that adhesive probiotic bacteria can inhibit pathogens attachment and help to remove them from the infected intestinal tract (Delcarlo et al., 2019). B0007 and B0008 to Caco-2 cell-line had adhesion index of 223 and 221 respectively. Past studies have shown B0007 with good adhesive features (Wang et al., 2014). The commercial probiotic isolate *L.acidophilus* LA-1 with Caco-2 cells has adhesion index of 155 (Bernet et al., 1994). The adhesion level of *L. fermentum* strain 4-17 to human colon adenocarcinoma Caco-2 cells was 8.5% (Falah et al., 2019). The observation that the B0010, B0011, B0024, B0077, B0106 and B0113 showed no adhesion to Caco-2 cells. The observation also obtained from that *L. bulgaricus* LB1 also showed no significant adhesion to either mucus-secreting or non-mucus-secreting cells (Gopal et al., 2001)

In this work, fermented mustard was used to isolate 50 LAB isolates. Nine of them were chosen based on their high activity of BSH. The nine LAB strains resisted gastrointestinal conditions well (pH, 3.0; bile salt, 0.5-1.0%). These isolates can have potential to be utilized in functional food and health products, due to their good probiotic properties mostly where the main aim is to reduce cholesterol in food. It is necessary to do *in vivo* study to confirm the effect of hypocholesterolemia.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

REFERENCES

Arepally D, Goswami TK (2019). Effect of inlet air temperature and gum Arabic concentration on encapsulation of probiotics by spray drying. LWT-Food Science and Technology 99:583-593.

Argyri AA, Zoumpopoulou G, Karatzas KAG, Tsakalidou E, Nychas GJN, Panagou EZ, Tassou CC (2013). Selection of potential probiotic lactic acid bacteria from fermented olives by in vitro tests. Food Microbiology 33:282-291.

Bernet MF, Brassart D, Neeser JR, Servin AL (1994). *Lactobacillus acidophilus* LA-1 binds to cultured human intestinalcell lines and inhibits cell-attachment and cell-invasionby enterovirulent bacteria. Gut 5:483-489.

Delcarlo SB, Parada R, Schelegueda IL, Vallejo M, Marguet ER, Campos CA (2019). From the isolation of bacteriocinogenic LAB strains to the application forfish paste biopreservation. LWT - Food Science and Technology 110:239-246.

Dunne C, O'Mahony L, Murphy L, Thornton G, Morrissey D, O'Halloran S, et al. (2001). *In vitro* selection criteria for probiotic bacteria of human origin: correlation with *in vivo* findings. The American Journal of Clinical Nutrition 73:386S-392S.

Falah F, Vasiee A, Behbahani BA, Yazdi FT, Roshanak S (2019). Evaluation of adherence and anti-infective properties of probiotic *Lactobacillus fermentum* strain 4-17 against *Escherichia coli* causing urinary tract infection in humans. Microbial Pathogenesis 31:246-253.

Gopal PK, Prasad J, Smart J, Gill HS (2001). *In vitro* adherenceproperties of *Lactobacillus rhamnosus* DR20 and *Bifido bacteriumlactis* DR10 strains and their antagonistic activityagainst an enterotoxigenic *Escherichia coli*. International Journal Food Microbiolology 67:207-216.

Guo LD , Wang LQ, Liu F, Li BL, Tang Y, Yu SF, Zhang DQ , Huo GC (2019). Effect of bile salt hydrolase-active *Lactobacillus plantarum* KLDS 1.0344 on cholesterol metabolism in rats fed a high-cholesterol diet. Journal of Functional Foods 61:1-6.

Guo XH, Kim JM, Nam HM, Park SY, Kim JM (2010). Screening lactic acid bacteria from swine origins for multistrain probiotics basedon *in vitro* functional properties. Anaerobe 16:321-326.

Lang GN, Binggeli C (2002). High-density lipoprotein restores endothelial function in hypercholesterolemic in men. Circulation 105:1399-1402.

Liong MT, Shal NP (2005a). Bile salt deconjugation ability, bile salt hydrolase activity and cholesterol co-precipitation ability of lactobacilli strains. International Dairy Journal 15:391-398.

Liong MT, Shal NP (2005b). Bile salt deconjugation and BSH activity of five bifidobacterial strains and their cholesterol co-precipitating properties.Food Research International 38:135-142.

Liong MT, Shal NP (2005c). Acid and bile tolerance and cholesterol removal ability of Lactobacilli strains. Journal of Dairy Science 88:55-66.

Lye HS, Rusul G, Liong MT (2010). Removal of cholesterol by lactobacilli via incorporation and conversion to coprostanol. Journal of Dairy Science 93:1383-1392

Michael AT, Christine LE, Douglas CY (2000). Molecular phylogenetic evidence for noninvasive zoonotic transmission of *Staphylococcus intermedius* from a canine pet to a human. Journal of Clinical Microbiology 38:1628-1631.

Miremadi F, Ayyash M, Sherkat F, Stojanovska L (2014). Cholesterol reduction mechanisms and fatty acid composition ofcellular membranes of probiotic *Lactobacilli* and *Bifidobacteria*. Journal of Functional Foods 9:295-305.

Mishra V, Prasad DN (2005). Application of *in vitro* methods for selection of *Lactobacillus casei* strains as potential probiotics. International Journal of Food Microbiology 103:109-115.

Nguyen TDT, Kang JH, Lee MS (2007). Characterization of *Lactobacillus plantarum* PH04, a potential probiotic bacterium with cholesterol-lowering effects. International Journal of Food Microbiology 113:358-361.

Pereira DI, Gibson GR (2002). Effects of consumption of probiotics and prebiotics on serum lipid levels in humans. Critical Reviews in Biochemistry and Molecular Biology 37:259-281.

Ramasamy K, Abdullah N, Wong CVL, Karuthan C, Ho YW (2010). Bile

salt deconjugation and cholesterol removal frommedia by *Lactobacillus* strains used as probiotics in chickens. Journal of the Science of Food and Agriculture 90:65-69.

Raza GS, Maukonen J, Makinen M, Niemi P, Niiranen L, Hibberd AA, Poutanen K, Buchert J, Herzig KH (2019). Hypocholesterolemic effect of the lignin-Rich insoluble residue of brewer's spent grain in mice fed a high-fat diet. Journal of Agricultural Food and Chemistry 67:1104-1114.

Reis SA, Conceição LL, Rosa DD, Siqueira NP, Peluzio MCG (2017). Mechanisms responsible for the hypocholesterolaemic effect of regular consumption of probiotics. Nutrition Research Reviews 30:36-49.

Shehata MG, El Sohaimy SA, El-Sahn, MA, Youssef MM (2016). Screening of isolated potential probiotic lactic acid bacteria for cholesterol lowering property and bilesalt hydrolase activity. Annals of Agricultural Sciences 61:65-75.

Sridevi N, Vishwe P,Prabhune A (2009). Hypocholesteremic effect of bile salt hydrolase from *Lactobacillus buchneri*ATCC4005. Food Research International 42:516-520.

Tanner MA, Everett CL, Youvan DC (2000). Molecular phylogenetic evidence for noninvasive zoonotic transmission of *Staphylococcus intermedius*from a canine pet to a human. Journal of Clinical Microbiology 38:1628-1631.

Tsai CC, Hsih HY, Chiu HH, Lai YY, Liu JH, Yu B, Tsen HY (2005). Antagonistic activity against *Salmonella* infection *in vitro* and *in vivo* for two *Lactobacillus* strains from swine and poultry. International Journal of Food Microbiology 102:185-194.

Vivek K, Mishra S, Pradhan RC (2019). Characterization of spray dried probiotic Sohiong fruit powder with *Lactobacillus plantarum*. LWT - Food Science and Technology 117:108699.

Wang SC, Chang CK, Chan SC, Shieh JS, Chiu CK, Duh PD (2014). Effects of lactic acid bacteria isolated from fermented mustard on loweringcholesterol. Asian Pacific Journal of Tropical Biomedicine 4:930-935.

Ward NC, Watts GF, Eckel RH (2019). Statin toxicity. Circulation Research 124:328-350.

Zhang M, Hang XM, Fan XB, Li DT, Yang H (2008). Characterization and selection of *Lactobacillu* strains for their effect on bile tolerance, taurocholatedeconjugation and cholesterol removal. World Journal of Microbiology Biotechnology 24:7-14.

AJ ACADEMIC JOURNALS
expand your knowledge

African Journal of Microbiology Research

Full Length Research Paper

Foodborne toxinfections caused by virus: Characteristics of the main viruses, prevention, treatment and clinical method of laboratory diagnosis by RT-qPCR

Karina Teixeira Magalhães-Guedes

Department of Bromatological Analysis, Pharmacy Faculty, Federal University of Bahia - UFBA, Barão of Geremoabo street, s/n, Ondina, CEP: 40171-970, Salvador, BA, Brazil.

Foodborne viruses were recognized among the top-rated food safety priorities and have become a greater concern to the food industry over the past few years. Food safety experts agreed that control measures for viruses throughout the food chain are required. This manuscript provides a description of foodborne viruses (Rotavirus, Adenovirus, Norovirus, Astrovirus, Hepatitis A, Hepatitis E, and Poliovirus) and their characteristics, and technologies developed for viral detection and control. A bibliographical research was carried out to collect data and information on viral diseases of the last nineteen years. The research sites accessed were: Books, theses and the database of Scielo, Google Scholar, Medline, Pubmed, Science Direct, CAPES periodical portal and virtual health library. This study was carried to clarify to the health professionals the importance of the need for a greater notification and update of these viral food toxinfections, in order to reduce the cases number of these diseases. Effective prevention and biological technologies to ensure control of viruses in the food chain can significantly reduce foodborne toxinfections caused by virus.

Key words: Enteric viruses, virus in food, quantitative reverse transcription polymerase chain reaction (RT-qPCR) in virus detection.

INTRODUCTION

Outbreaks and diseases caused by microorganisms in food, in particular viruses, pose a major health problem, not only because they cause disease, but also through the costs associated with measures taken to reduce impacts on world populations. In today's world with its global reach, the potential for the spread of foodborne viral diseases on all continents is immense (CDC, 2016; MDS, 2018).

Viruses are inert particles when they are outside their hosts, and their associated risk depends on maintaining their ability to become infected by causing diseases (Trabulsi and Alterthum, 2008). In the viral constitution

E-mail: karynamagat@gmail.com. Tel: +55 (71) 3283-6920.
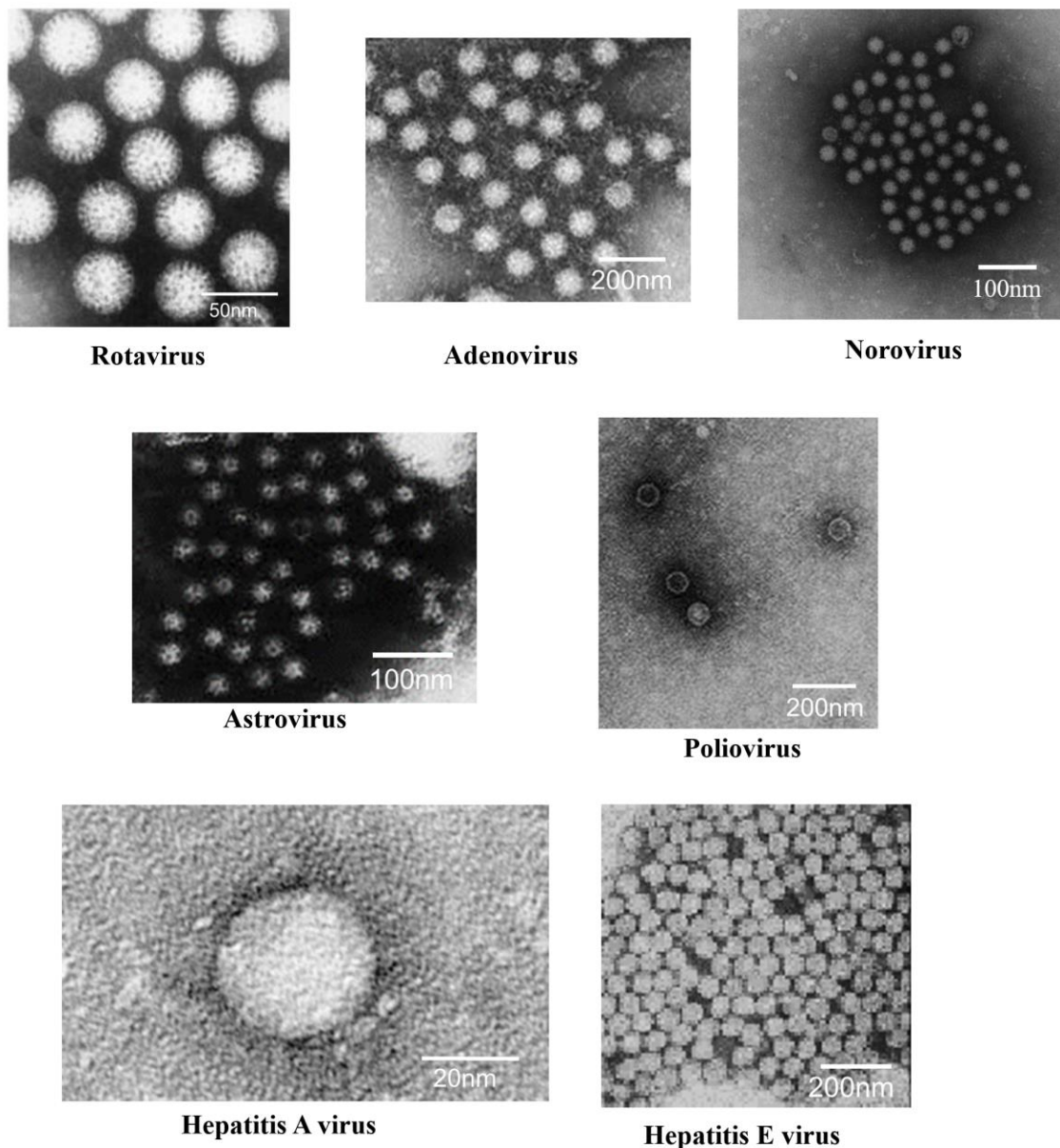
Figure 1. Electron micrograph of viral particles causing foodborne toxinfections. Created by the author. Negative staining technique by direct electron microscopy 33000 X increase.

there is usually only one type of nucleic acid.

Single stranded or double stranded DNA or RNA. Viruses also have proteins, glycoproteins and/or glycolipids (Trabulsi and Alterthum, 2008).

Foodborne toxinfections caused by virus is predominantly transmitted via fecal-oral through ingestion of contaminated food and/or water or through a secondary route of infection and/or person-to-person contact. The major pathogens causing viral foodborne diseases are Rotavirus, Enteric Adenovirus, Norovirus, Astrovirus, Hepatitis A virus, Hepatitis E virus, and Poliovirus (Figure 1) (Xavier et al., 2009; Kokkinos et al., 2015; Polo et al., 2015; Jacxsens et al., 2017; Müller et

al., 2017; Sarno et al., 2017; Donia, 2018; Liu et al., 2018). A large number of these viral particles may be found in the human gastrointestinal tract samples (Table 1).

The ways to prevent foodborne toxinfections caused by virus are by applying good personal hygiene practices, utensils, proper food hygiene and proper water treatment for cleaning and consumption. (Polo et al., 2015; MDS, 2018). Foodborne toxinfections caused by virus needs to be published so that the community knows the ways of contagion, prevention, diagnosis and treatment of these foodborn toxinfections.

Most methods currently used for the detection of

Table 1. Viral particles found in human gastrointestinal tract samples.

Genus	Genome	Popular name	Disease caused	Reference
Enterovirus	ssRNA	Poliovirus	Paralysis, meningitis, fever	Donia (2018); Liu et al. (2018)
Hepatovirus	ssRNA	Hepatitis A virus	Hepatitis	Costafreda et al. (2006); Liu et al. (2018)
Rotavirus	Segmented dsRNA	Rotavirus	Gastroenteritis	Bwogi et al. (2016); Liu et al. (2018)
Hepevirus	ssRNA	Hepatitis E virus	Hepatitis	Liu et al. (2018)
Mamastrovirus	ssRNA	Astrovirus	Gastroenteritis	Liu et al. (2018)
Norovirus	ssRNA	Norovirus	Gastroenteritis	Ozawa et al. (2007); Liu et al. (2018)
Mastadenovirus	dsDNA	Adenovirus	Gastroenteritis	Liu et al. (2018)

foodborne viruses are based on polymerase chain reaction (PCR) techniques and variants. Recent technical developments offer opportunities to improve detection, quantification and identification of foodborne viruses such as RT-qPCR. Closed-loop detection of the RT-qPCR assay can effectively prevent and reduce the incidence of false positives (Sedlak and Jerome, 2013; Zhao et al., 2013; Jia et al., 2019).

The objectives of this review constitute the purpose of an academic study, intended to contribute to the knowledge of the main foodborne toxinfections caused by virus, its transmission, prevention, treatment and clinical methods of laboratory diagnosis. A bibliographic survey on foodborn toxinfections caused by virus worldwide was carried out to clarify to health professionals the importance of the need for greater notification and updating of foodborn toxinfections caused by virus, in order to reduce the number of cases of these foodborne toxinfections.

METHODOLOGY

A bibliographic research study was conducted to collect data and information on foodborne illnesses of viral origin from the last nineteen years. The research sites accessed were: e-books, theses and the Scielo database, Google Scholar, Medline, Pubmed, Science Direct and CAPES periodical portal and the virtual health library. The index terms used for single searches were Rotavirus, Adenovirus, Norovirus, Astrovirus, Hepatitis A virus, Hepatitis E virus, and Poliovirus. The combined search for words were viral foodborne diseases, viral gastroenteritis, viral foodborne infections, clinical methods of laboratory diagnosis of foodborn toxinfections caused by viruses. The term "RT-qPCR in virus detection" was searched. The justification is as follows: When qPCR is coupled with a preceding reverse transcription reaction, it can be used to quantify gene expression (RT-qPCR). qPCR has been shown to be a robust, highly reproducible and sensitive method to quantitatively track phylogenetic and functional gene changes across temporal and spatial scales under varying environmental or experimental conditions. The provision of qPCR data sets that describe the abundance of specific bacterias, yeasts and viruses or genes to complement other quantitative environmental data sets is of increasing importance in microbial ecology as it furthers understanding of the roles and contributions of particular microbial and functional groups within ecosystem functioning.

Furthermore, reverse transcription (RT) analyses are now increasingly combined with Q-PCR methods in RT-qPCR assays, offering a powerful tool for quantifying gene expression (in terms of numbers of rRNA and mRNA transcripts) and relating biological activity to ecological function of viruses.

Papers that did not match the searched words were excluded, as well as those that did not fit in the pre-selected years from 2000 to 2019. The exclusion criterion also applies to articles that after reading that did not refer to the main objective of the study. In total 39 articles were recruited, among them 34 in English and 4 in Portuguese.

DEVELOPMENT

Viruses that cause foodborne toxinfections

Rotavirus

A genus of double-stranded ribonucleic acid (RNA) viruses, belonging to the Reoviridae family, are predominantly transmitted fecal-orally and have been found in various foods (Kittigul et al., 2015; Jones and Muehlhauser, 2017). Rotavirus is a virus that causes infectious gastroenteritis and mortality in infants and children worldwide, especially in children under 5 years of age, despite its occasional occurrence as pathogens in adolescents and adults (Mizukoshi et al., 2015; Bwogi et al., 2016). There is no specific medicine to treat rotavirus infections. Nonspecific antiviral drugs and administration of a variety of fluids are used to alleviate clinical symptoms for rotavirus infections (Nan et al., 2014; Liu et al., 2018).

Transmission may occur through ingestion of contaminated water and/or food, person-to-person contact, contaminated objects, and respiratory secretions (Jones and Muehlhauser, 2017). The main symptoms caused by rotavirus toxinfection are diarrhea, vomiting, nausea, anorexia, cramps and malaise (Nan et al., 2014; Bwogi et al., 2016; Liu et al., 2018).

Rotavirus toxinfection is diagnosed by direct detection of rotavirus virus in the stool. Prevention is accomplished by hygienic measures such as proper hand washing and proper water treatment (Nan et al., 2014; Bwogi et al., 2016; Liu et al., 2018). The main treatment for rotavirus toxinfection is oral rehydration (Nan et al., 2014; Bwogi et al., 2016; Liu et al., 2018). Another form of prevention is vaccination. Rotavirus vaccination is part of the Brazilian immunization calendar and round the world. The vaccine is given free of charge to the population in two doses at 2 and 4 months of age (Brasil, 2009).

Adenovirus

It is a family of envelopeless icosahedral double-stranded deoxyribonucleic acid (ADN) viruses with diameters ranging from 65 to 80 nm (Hur et al., 2013; Liu et al., 2018). Adenoviruses usually cause mild infections involving the upper or lower respiratory tract, gastrointestinal tract, or conjunctiva (Hur et al., 2013). Enteric adenovirus infection can increase appetite and food intake, lowering leptin hormone levels (effect on appetite control and body mass), increasing the prevalence of obesity (Hur et al., 2013; Liu et al., 2018).

Enteric adenovirus infection has the symptoms of diarrhea, vomiting and fever. Diagnosis is made by isolating the adenovirus virus in cell culture or by detecting an increase in the number of antibodies (Hur et al., 2013; Liu et al., 2018). There is no antiviral therapy. There are vaccines only against the adenovirus virus that reaches the respiratory tract. Prevention is done through good sanitary conditions and by personal hygienic measures. The main treatment of infection is fluid and electrolyte replacement to prevent dehydration (Hur et al., 2013; Liu et al., 2018).

Astrovirus

Viruses belonging to the *Astroviridae* family, comprising two genera: *Mamastrovirus* and *Avastrovirus*. *Mamastrovirus* include astrovirus that infect humans and animals. *Avastrovirus* includes astrovirus that infect birds (Jeong et al., 2012). Astrovirus is classified into eight classic human types (HAstV-1/8) and seven other less prevalent types, described as HAstV VA1, VA2, VA3, VA4, MLB-1, MLB-2, and MLB-3. Astrovirus has three protein icosahedral capsid and is made up of double-stranded RNA (Jeong et al., 2012).

During outbreaks by astrovirus, the elderly and children are the most affected, and the spread of the virus is associated with person-to-person contact, food intake and contaminated water. Astrovirus infection has as its main symptom mild watery diarrhea, headache, nausea, vomiting and general malaise (Jeong et al., 2012). The most serious infected may also have anorexia, abdominal pain, fever and mild dehydration (Medici et al., 2015).

Diagnosis of astrovirus toxinfection is made by detecting viral particles or specimens in the stool or rectal swabs (Jeong et al., 2012). Preventing astrovirus toxinfection is sanitation, eating well-cooked food, and personal hygiene of food and utensils (Jeong et al., 2012). There is no vaccine or antiviral therapy against Astrovirus and treatment is to prevent dehydration or rehydrate the patient in more severe cases (Jeong et al., 2012; Medici et al., 2015).

Norovirus

Norwalk virus is the human norovirus. Noroviruses are made up of a capsid and a nucleic acid, measuring about 27 to 30 nm in diameter. They have no wrap. The nucleocapsid is rounded and exhibits icosahedral symmetry (Ozawa et al., 2007). The viral genome consists of a linear single-stranded positive-polarity RNA molecule. Genomes with these characteristics serve as mRNA. Viral particles penetrate the target cell they bind to cellular ribosomes and protein translation occurs (Ozawa et al., 2007).

Noroviruses are the leading cause of outbreaks of gastroenteritis in the world. These viruses cause outbreaks in including schools, cruise ships and restaurants. Noroviruses have been detected in environmental samples and foods such as salads, shellfish, sandwiches and fruits (Yamashita et al., 2001; Lee et al., 2007; Tu et al., 2008).

The main route of transmission is oral fecal through person-to-person contact. Norovirus is extremely infectious and has a resistance that allows it to remain on infected surfaces. This results in a problem with the use of shared objects and collective spaces. Norovirus can be spread through saliva particles or contaminated water, and infection is also strongly associated with poor hygiene (no hand washing) (Ozawa et al., 2007). The most widely used method of detecting Norovirus is RT-qPCR, which has high sensitivity (Vinjé et al., 2004; Ozawa et al., 2007; Liu et al., 2018).

Hepatitis A virus

Hepatitis A virus is 27 nm in size and belongs to the *Picornavirus* family, such as the polio virus. The hepatitis A virus genome is composed of RNA and has no envelope (Costafreda et al., 2006; Liu et al., 2018). Hepatitis A virus is found all over the world, especially in places where hygiene conditions are poor. The virus spreads through the fecal-oral route through water, contaminated food and objects and by person-to-person contact. There are rarely cases of percutaneous (accidental inoculation) and parenteral (transfusion) transmission of direct or indirect contact with fecal material. Hepatitis A virus has 7 genotypes named I through VII with genotypes I, II, III and IV capable of infecting humans (Costafreda et al., 2006).

The hepatitis A virus settles in the intestinal mucosa. Later the hepatocytes are infected by the virus from the primary viremia (Costafreda et al., 2006; MDS, 2018). The virus replicates in hepatocytes generating inflammation in the liver by activating the immune system. The virus is excreted in faeces and can survive under environmental conditions for a long time. Astrovirus toxinfection has mild water diarrhea, headache, nausea, vomiting and general malaise as symptoms (Costafreda et al., 2006). In more severe cases the infected person may also have anorexia, abdominal pain, fever and dehydration (Costafreda et al., 2006).

Hepatitis A virus transmission occurs fecally-orally, through intimate contact with infected persons, and from drinking contaminated food and water (Costafreda et al., 2006). The diagnosis of the disease is made by detecting viral particles in the stool and/or rectal swabs. Prevention against Astrovirus toxification is basic sanitation, eating well-cooked food and proper hygiene of hands, food and utensils (MDS, 2018). There is no vaccine or antiviral therapy against hepatitis A virus. Treatment consists of preventing dehydration or rehydrating more severe patients (MDS, 2018).

Hepatitis E virus

Hepatitis E virus is small (27-34 nm) not enveloped with an RNA genome. Hepatitis E virus belongs to family *Hepeviridae* (Lu et al., 2006; Ahmad et al., 2011). Hepatitis E is a toxicity transmitted by ingestion of the virus present in contaminated water and food and/or by human and animal waste. Transmission can also occur vertically (from mother to child) (Lu et al., 2006). The virus replicates first in the gastrointestinal tract and then migrates to the liver by multiplying in hepatocytes. Virus particles are excreted in faeces (Lu et al., 2006; Ahmad et al., 2011). The main symptoms of infection are abdominal pain, nausea, vomiting, anorexia and jaundice (Lu et al., 2006; Ahmad et al., 2011). Hepatitis E can happen asymptomatically (Krawczynski et al., 2011). Mortality is low, but in pregnant women mainly in the third trimester of pregnancy this percentage can reach 25% (Ahmad et al., 2011; Krawczynski et al., 2011). Hepatitis E is diagnosed by the detection of antibodies (immunoglobulin M [IgM] and immunoglobulin G [IgG]) in serum. There is no vaccine marketed for hepatitis E. Usually the cure of the disease is spontaneous, but treatment is suggested rest and prohibition of alcohol use (Ahmad et al., 2011). Prevention is through adequate public hygiene and sanitation conditions (Ahmad et al., 2011).

Poliovirus

Poliovirus belongs to family Picornaviridae. Polovirus is the agent that causes polio in humans. It is a simple virus with only one single stranded RNA without envelope and with a 30nm icosahedral protein capsid (Donia, 2018; Liu et al., 2018).

Poliovirus is usually transmitted via the fecal-oral route (by water, contaminated food or objects), the direct route (person-to-person) and/or orally by droplets of secretions when speaking, coughing or sneezing (Donia, 2018). The poliovirus replicates in the oropharynx and subsequently migrates to the small intestine. In paralytic poliomyelitis, poliovirus is spread in the bloodstream to the central nervous system, mainly destroying motor neurons, causing muscle weakness and flaccid paralysis in the patient. Polio lethality ranges from 2 to 10% (Donia, 2018).

Paralytic forms of polio are uncommon, occurring in 1 to 1.6% of cases. When polio is symptomatic, it is divided into three classes: First class absorptive polio there is the appearance of fever without signs of localization in the central nervous system. In the second class there is the onset of fever and aseptic meningitis with rapid and complete recovery. In the third class paralytic polio the infected presents fever associated with meningeal irritation and asymmetrical flaccid paralysis. In the affected parts arise cramps and muscle spasms. In acute paralytic poliomyelitis toxinfection poliovirus invades the central nervous system generates a total or partial injury to spinal motor neurons disrupting some innervations of muscle fibers causing flaccid paralysis (Donia, 2018). People who have had paralytic poliomyelitis may have symptoms of neurological disorders, weakness, fatigue, muscle atrophy, muscle and joint pain, sleep disturbance, cold intolerance, breathing and swallowing difficulty, and weight gain. These symptoms are referred to as "post polio syndrome" (Donia, 2018).

Polio vaccines are vaccines used to prevent poliomyelitis (polio). Two types are used: an inactivated poliovirus given by injection (IPV) and a weakened poliovirus given by mouth (OPV). World Health Organization (WHO) recommends all children be fully vaccinated against polio. The two vaccines have eliminated polio from most of the world, and reduced the number of cases reported each year from an estimated 350,000 in 1988 to 33 in 2018 (MDS, 2018). In Brazil the polio vaccine is part of the vaccination schedule. The vaccine is distributed free and administered in four doses at 2 months, then 4 months, 6 months and 15 months of life (booster vaccine) (MDS, 2018). Diagnosis is made by isolating the poliovirus in throat material, feces or by raising antibodies. Quarantine those infected with poliovirus is ineffective because excretion of poliovirus in faeces occurs before symptoms appear. There is no antiviral therapy for polio. Treatment is based on relieving symptoms, assisting breathing and physiotherapy of the affected muscles (MDS, 2018).

Virus detection in food

Cell culture based methods can be used to detect some

Table 2. Foods most commonly related to outbreaks in each type of virus.

Virus	Foods	References
Norovirus	Frozen raspberries[a], leafy green vegetable[a], berry fruit[a], drinking water[b], seafood[a], lettuce[a], prawns[a]	Sumner (2011), Bouwknegt et al. (2015), Jacxsens et al. (2017)
Poliovirus	Chicken[a], prawns[a]	Golden et al. (2009)
Hepatitis A virus	Leafy green vegetable[a], berry fruit[a], drinking water[b], seafood[b], lettuce[a], prawns[a]	Sumner (2011); Bouwknegt et al. (2015); Jacxsens et al. (2017)
Rotavirus	Prawns[a], fish[a]	Sumner (2011)
Hepatitis E virus	Leafy green vegetable[a], berry fruit[a], drinking water[b], seafood[a], lettuce[a], pork and wild boar products[b]	Bouwknegt et al. (2015); Jacxsens et al. (2017); Müller et al. (2017)
Astrovirus	Chicken[a], prawns[a]	Golden et al. (2009); Sumner (2011)
Adenovirus	Chicken[a], prawns[a]	Golden et al. (2009); Sumner (2011)

[a]High incidence of outbreaks, [b] Moderate incidence of outbreaks.

foodborne viruses using a series of concentration and purification steps to elute the food matrix virus taking special care to avoid reducing virus infectivity. Table 2 shows the foods most commonly related to outbreaks in each type of virus.

Cell culture-based methods have been used to initially amplify viral nucleic acids and remove inhibitors prior to detection of RT-qPCR. This RT-qPCR assay has been used to detect Rotavirus, Adenovirus, Norovirus, Astrovirus, Hepatitis A, Hepatitis E, and Poliovirus in food (Sánchez et al., 2012; Stals et al., 2013; Zhao et al., 2013; Perrin et al., 2015; Bwogi et al., 2016; Donia, 2018; Liu et al., 2018; Jia et al., 2019).

Virus quantification represents a breakthrough in outbreak investigations and routine monitoring as it can provide data to develop food acceptance levels and the development of quantitative risk assessments (Sánchez et al., 2012). qPCR quantitation can be done using a standard curve generated from known quantities of the target sequence represented by synthetic or *in vitro* transcribed DNA or RNA (Sánchez et al., 2012; Stals et al., 2013; Zhao et al., 2013; Perrin et al., 2015; Bwogi et al., 2016; Donia, 2018; Liu et al.,

2018; Jia et al., 2019). Regardless of the method used, the most critical step is the reverse transcription reaction (RT) (Vimont et al., 2015). Sensitive and quantitative detection of foodborne enteric viruses is classically achieved by quantitative RT-PCR (RT-qPCR). Recently, digital PCR (dPCR) was described as a novel approach to genome quantification without need for a standard curve. The performance of microfluidic digital RT-PCR (RT-dPCR) was compared to RT-qPCR for detecting the main viruses responsible for foodborne outbreaks (human Noroviruses) and Hepatitis A virus in spiked lettuce and bottled water. Two process controls (Mengovirus and Murine Norovirus) were used and external amplification controls were added to examine inhibition of RT-qPCR and RT-dPCR (Vimont et al., 2015).

However, virus quantification may vary with the use of different standard materials by each analytical laboratory. This suggests that the use of certified standard reagents may reduce the variation (Vimont et al., 2015). Importantly, viruses are often unevenly distributed in a food batch making it necessary to test replicates or a set of

samples for reliable qualitative or quantitative results (Sánchez et al., 2012; Stals et al., 2013; Zhao et al., 2013; Perrin et al., 2015; Bwogi et al., 2016; Donia, 2018; Liu et al., 2018; Jia et al., 2019). There are currently no regulatory microbiological criteria (eg. standards, guidelines or specifications) applied to viruses. Most food companies and authorities mainly request qualitative results as part of production hygiene tests or outbreak investigations (MDS, 2018). To confirm a positive virus presence signal by RT-qPCR and to assist epidemiological studies, systematic typing of disease outbreak strains and virus surveillance in "healthy" foods without viral contamination is recommended (Sánchez et al., 2012; Stals et al., 2013; Zhao et al., 2013; Perrin et al., 2015; Bwogi et al., 2016; Donia, 2018; Liu et al., 2018; Jia et al., 2019).

Control and prevention of foodborne diseases

There is a growing demand for food safety information at the international, national and local level. The following are information about control

and prevention of foodborne diseases (Brasil, 2009).

(i) Wash your hands before handling food and often during food preparation
(ii) Wash your hands after going to the toilet
(iii) Wash and sanitize all surfaces and equipment used for food preparation
(iv) Protect kitchen areas and food from insects, pests and other animals
(v) Separate raw meat, poultry and seafood from other foods
(vi) Use separate equipment and utensils such as knives and cutting boards for handling raw foods
(vii) Store food in containers to avoid contact between raw and prepared foods
(viii) Cook food thoroughly, especially meat, poultry, eggs and seafood
(ix) Bring foods like soups and stews to boiling to make sure that they have reached 70°C. For meat and poultry, make sure that juices are clear, not pink. Ideally, use a thermometer
(x) Reheat cooked food; thoroughly keep food at safe temperatures
(xi) Do not leave cooked food at room temperature for more than 2 h
(xii) Refrigerate promptly all cooked and perishable food (preferably below 5°C)
(xiii) Keep cooked food piping hot (more than 60°C) prior to serving
(xiv) Do not store food too long even in the refrigerator
(xv) Do not thaw frozen food at room temperature
(xvi) Use safe water or treat it to make it safe
(xvii) Select fresh and wholesome foods
(xviii) Choose foods processed for safety, such as pasteurized milk
(xix) Wash fruits and vegetables, especially if eaten raw
(xx) Do not use food beyond its expiry date.

Future trends in viral food safety

Given the importance of foodborne viruses and the impact that different factors (globalisation of the market, increased international travel, consumer demands, changes in food-processing, pathogen evolution, etc.) may have on the emergence of disease, it is clear that priority needs to be given to expanding foodborne disease surveillance to cover foodborne viruses. The expansion should include a more complete coverage of qualified laboratories per country. Inclusion of more countries in international surveillance networks and development and implementation of detection methods of more classes of viruses in the surveillance programs (Brasil, 2009).

Virus detection in clinical samples

The diagnosis of viral infections has emerged in recent

decades as an important tool in medicine effectively contributing to the identification of the pathogen directing the treatment of the disease (Tavares et al., 2005; Sedlak and Jerome, 2013; MDS, 2018). Until recently, virus diagnosis was not performed in clinical laboratories or hospitals because the techniques used were very slow and expensive, reagents were not available and there was no treatment for viral infections limiting the use of diagnostic tests (Sedlak and Jerome, 2013; Wylie et al., 2018).

Laboratory diagnosis of viruses has been divided into classical diagnosis including serology and rapid diagnosis of viruses aimed at direct demonstration of virus, antigens or viral nucleic acids in clinical specimens. Some techniques for virus identification are not immunological and do not depend on antigen and antibody binding. These techniques are based on the molecular biology of viruses specifically in identifying unique nucleic acid sequences. Molecular biology techniques are essential tools for viral disease detection, viral load monitoring, antiviral therapy monitoring and genotyping of various viruses (Liu et al., 2018). The development of RT-qPCR allowed the application of a quantitative method in the laboratory diagnosis of viral infections (Donia, 2018; Wylie et al., 2018). Quantitative PCR (qPCR) is used to detect, characterize and quantify nucleic acids for numerous applications. Commonly, in RT-qPCR, RNA transcripts are quantified by reverse transcribing them into cDNA first, as described above and then qPCR is subsequently carried out. As in standard PCR, DNA is amplified by 3 repeating steps: denaturation, annealing and elongation. However, in qPCR, fluorescent labeling enables the collection of data as PCR progresses. This technique has many benefits due to a range of methods and chemistries available (e.g. dye-based qPCR (typically green) and probe-based qPCR (Donia, 2018; Wylie et al., 2018).

Currently, in all the world, another technique presents a remarkable demand to identify viruses in clinical specimens in a fast selective and accurate manner, the use of biosensors (Mokhtarzadeh et al., 2017). Several biosensors have been designed and marketed for detection of pathogenic viruses in clinical specimens. However, they present many challenges in the efficiency of results (Mokhtarzadeh et al., 2017). Nanotechnology overcomes these challenges and performs direct detection of molecular targets in real time. In this overview studies on nanotechnology-based biosensors for detection of pathogenic viruses are being studied and applied in clinical sample analysis. Technologies include nanotubes based on nanotubes (carbon, gold, silver and zinc) and magnetic nanoparticles (Mokhtarzadeh et al., 2017).

Conclusion

Based on the results of this research it was concluded

that: food viruses occupy a prominent place causing major problems in the health of the world population. Through this bibliographic research it was possible to verify the different toxin infections caused by viruses in foods showing that there is a need for greater notification of these food viruses in order to reduce the cases number of these pathogens. The spread of the virus is due to inadequate sanitary conditions since the main route of transmission of these infections is the fecal-oral route and the developing countries are the hardest hit. Prevention against food contamination is necessary by ensuring the control of viral transmissions significantly reducing toxinfections.

The RT-qPCR methodology offers an advantage of using molecular biology to detect different types of viruses in clinical and food samples. Technological improvements to biological protocols, instruments, and strategies drive greater popularity of the RT-qPCR methodology for virus detection in clinical and food samples. It is hoped that in the near future all technologies applied for virus detection in clinical samples may also be effective in detecting pathogenic viruses in food samples.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

REFERENCES

Ahmad I, Holla RP, Jameel S (2011). Molecular virology of hepatitis E virus. Virus Research 161(1):47-58.

Bouwknegt M, Verhaelen K, Rzeżutka A, Kozyra I, Maunula L, von Bonsdorff C-H, Vantarakis A, Kokkinos P, Petrovic T, Lazic S (2015). Quantitative farm-to-fork risk assessment model for norovirus and hepatitis A virus in European leafy green vegetable and berry fruit supply chains. International Journal of Food Microbiology 198:50-58.

Brasil (2009). Guia de vigilância epidemiológica / Ministério da Saúde, Secretaria de Vigilância em Saúde, Departamento de Vigilância Epidemiológica. – 7. ed. – Brasília. Ministério da Saúde 816 p. – (Série A. Normas e Manuais Técnicos).

Bwogi J, Malamba S, Kigozi B, Namuwulya P, Tushabe P, Kiqui S, Byarugaba DK, Desselberger U, Iturriza-Gomara M, Karamagi C (2016). The epidemiology of rotavirus disease in under-five-year-old children hospitalized with acute diarrhea in central Uganda, 2012-2013. Archives of Virology 161(4):999-1003.

Centers for Disease Control and Prevention (CDC) Norovirus worldwide (2016) Disponível em: <https://www.cdc.gov/norovirus/worldwide.html>. Acesso em: 19 set. 2019.

Costafreda MI, Bosch A, Pintó RM (2006). Development, evaluation, and standardization of a real-time TaqMan reverse transcription-PCR assay for quantification of hepatitis A virus in clinical and shellfish samples. Applied and Environmental Microbiology 72:3846-3855.

Donia DT (2018). qRT-PCR for enterovirus detection: Conversion to ultrafast protocol. Journal of King Saud University Science 30(2):180-184.

Golden NJ, Schlosser WD, Ebel ED (2009). Risk assessment to estimate the probability of a chicken flock infected with H5N1 highly pathogenic avian influenza virus reaching slaughter undetected. Foodborne Pathogens Disease 6:827-835.

Hur SJ, Kim DH, Chun SC, Lee SK (2013). Effect of adenovirus and influenza virus infection on obesity. Life Sciences 93(16):531-535.

Jacxsens L, Stals A, De Keuckelaere A, Deliens B, Rajkovic A,

Uyttendaele M (2017). Quantitative farm-to-fork human norovirus exposure assessment of individually quick-frozen raspberries and raspberry puree. International Journal of Food Microbiology 242:87–97.

Jeong HS, Jeong A, Cheon D-S (2012). Epidemiology of astrovirus infection in children. Korean Journal of Pediatrics 55(3):77-82.

Jia S, Feng B, Wang Z, Ma Y, Gao X, Jiang Y, Cui W, Qiao X, Tang L, Li Y, Wang L, Xu Y (2019). Dual priming oligonucleotide (DPO)-based real-time RT-PCR assay foraccurate differentiation of four major viruses causing porcine viral diarrhea. Molecular and Cellular Probes 47:1-10.

Jones TH, Muehlhauser V (2017). Frequency of hepatitis E virus, rotavirus and porcine enteric calicivirus at various stages of pork carcass processing in two pork processing plants. International Journal of Food Microbiology 259(16):29-34, 2017.

Kittigul L, Singhaboot Y, Chavalitshewinkoon-Petmitr P, Pombubpa K, Hirunpetcharat C (2015). A comparison of virus concentration methods for molecular detection and characterization of rotavirus in bivalve shellfish species. Food Microbiology 46:161-167.

Kokkinos P, Bouwknegt M, Verhaelen K, Willems K, Moloney R, Roda Husman AM, D'Agosyino M, Cook N, Vantarakis A (2015). Virological fit-for-purpose risk assessment in a leafy green production enterprise. Food Control 51:333-339.

Krawczynski K, Meng XJ, Rybczynska J (2011). Pathogenetic elements of hepatitis E and animal models of HEV infection. Virus Research 161(1):78-83.

Lee N, Chan MC, Wong B, Choi KW, Sin W, Lui G, Chan PK, Lai RW, Cockram CS, Sung JJ, Leung WK (2007). Fecal viral concentration and diarrhea in norovirus gastroenteritis. Emerging Infectious Disease Journal 13:1399-401.

Liu P, Lu L, Xu M, Zhong H, Jia R, Su L, et al. (2018). A novel multiplex PCR for vírus detection by melting curve analysis. Journal of Virological Methods 262:56-60.

Lu L, Li C, Hagedorn CH (2006). Phylogenetic analysis of global hepatitis E virus sequences: genetic diversity, subtypes and zoonosis. Reviews Medical Virology 16(1):5-36.

MINISTÉRIO DA SAÚDE (MDS). Doenças transmitidas por alimentos (2018). Disponível em: <https:www.portalms.saude.gov.br/saude-de-a-z/doencas-transmitidas-por-alimentos>. Acesso em: 25 set. 2019.

Medici MC, Tummolo F, Martella V, Banyai K, Bonerba E, Chezzi C, Arcangeletti MC, De Conto F, Calderaro A (2015). Genetic heterogeneity and recombination in type-3 human astroviruses. Infection. Genetics and Evolution Journal 32:156-160.

Mizukoshi K, Kuroda M, Tsukaqoshi H, Sekizuka T, Funatoqawa K, Morita Y, Noda M, Katayama K, Kimura H (2015). A food-borne outbreak of gastroenteritis due to genotype G1P[8] rotavirus among adolescents in Japan. Microbiology and Immunology 58(9):536-539.

Mokhtarzadeh A, Eivazzadeh-Keihan R, Pashazadeh P, Hejazi M, Gharaatifar N, Hasanzadeh M, Baradaran B, de la Guardia M (2017). Nanomaterial-based biosensors for detection of pathogenic virus. TrAC Trends in Analytical Chemistry 97:445-457.

Müller A, Collineau L, Stephan R, Müller A, Stärk KDC (2017). Assessment of the risk of foodborne transmission and burden of hepatitis E in Switzerland. International Journal of Food Microbiology 242:107-115.

Nan X, Jinyuan W, Yan Z, Maosheng S, Honqujun L (2014). Epidemiological and clinical studies of rotavirus-induced diarrhea in China from1994–2013. Human Vaccines and Immunotherapeutics 10(12):3672-3680.

Ozawa K, Oka T, Takeda N, Hansman GS (2007). Norovirus infections in symptomatic and asymptomatic food handlers in Japan. Journal of Clinical Microbiology 45:3996-4005.

Perrin A, Loutreul J, Boudaud N, Bertrand I, Gantzer C (2015). Rapid, simple and efficient method for detection of viral genomes on raspberries. Journal of Virological Methods 224:95-101.

Polo D, Feal X, Romalde JL (2015). Mathematical model for viral depuration kinetics in shellfish: an useful tool to estimate the risk for the consumers. Food Microbiology 49:220–225.

Sánchez G, Elizaquível P, Aznar R (2012). Discrimination of Infectious Hepatitis A Viruses by Propidium Monoazide Real-Time RT-PCR. Food and Environmental Virology 4(1):21-25.

Sarno E, Martin A, McFarland S, Johne R, Stephan R, Greiner M

(2017). Estimated exposure to hepatitis E virus through consumption of swine liver and liver sausages. Food Control 73:821-828.

Sedlak RH, Jerome KR (2013). Viral diagnostics in the era of digital polymerase chain reaction. Diagnostic Microbiology and Infectious Disease 75:1-4.

Stals A, Van Coillie E, Uyttendaele M (2013). Viral genes everywhere: public health implications of PCR-based testing of foods. Current Opinion in Virology 3(1):69-73.

Sumner J (2011). Food Safety Risks Associated with Prawns Consumed in Australia. http://australianwildprawns.com.au/wp-content/uploads/2017/02/2009-787-Foodsafety-risks-for-prawns.pdf

Tavares TM, de Paula Cardoso DD, Brito WMED (2005). Vírus entéricos veiculados por água: aspectos microbiológicos e de controle de qualidade da água. Revista de Patologia Tropical 34(2):85-104.

Trabulsi LR, Alterthum F (2008). Microbiologia. 5. ed. São Paulo: Atheneu 718 p.

Tu ET, Bull RA, Kim MJ, Mc Ivir CJ, Heron I, Mc Iver CJ, Heron L, Rawlinson WD, White PA (2008). Norovirus excretion in an aged-care setting. Journal of Clinical Microbiology 46:2119-121.

Vimont A, Fliss I, Jean J (2015). Efficacy and Mechanisms of Murine Norovirus Inhibition by PulsedLight Technology. Applied and Environmental Microbiology 81(8):2950-2957.

Vinjé J, Hamidjaja RA, Sobsey MD (2004). Development and application of a capsid VP1 (region D) based reverse transcription PCR assay for genotyping of genogroup I and II Noroviruses. Journal of Virological Methods 116:109-117.

Wylie K.M, Wylie TN, Buller R, Herter B, Cannella MT, Storch GA (2018). Detection of viruses in clinical samples by use of metagenomic sequencing and targeted sequence capture. Journal of Clinical Microbiology 56(12):1111-1123.

Xavier MPTP, Oliveira SA, Ferreira MSR, Victoria M, Miranda V, Silva MFM, Strina A, Barreto ML, Miagostovicht MP, Leite JPG (2009). Detection of caliciviruses associated with acute infantile gastroenteritis in Salvador, an urban center in Northeast Brazil. Brazilian Journal of Medical and Biological Research 42:438-444.

Yamashita T, Ito M, TsuzukI H, Sakae K (2001). Identification of Auchi virus infection by measurement of immunosorbent assay. Journal of Clinical Microbiological 39:4178-4180.

Zhao J, Shi BJ, Huang XG, Peng MY, Zhang XM, He DN, Pang R, Zhou B, Chen PY (2013). A multiplex RT-PCR assay for rapid and differential diagnosis of four porcine diarrhea associated viruses in field samples from pig farms in East China from 2010 to 2012. Journal of Virological Methods 194:107-112.

AJ ACADEMIC JOURNALS
expand your knowledge

African Journal of Microbiology Research

Full Length Research Paper

Virulence characteristics and phylogenetic background of antibiotic-resistant *Enterococcus faecalis* from abattoir, poultry and clinical origin in Ado-Ekiti, Nigeria

Abiodun Ojo[1,2*], Adetunji Olawale[2], Pius Okiki[2], Folusho Oyinloye[2], Tolulope Ogunnusi[2], Amos Onasanya[3] and Oyewale Morakinyo[4]

[1]Malaria Consortium, Abuja, Nigeria.
[2]Department of Biological Sciences, College of Sciences, Afe Babalola University, Ado-Ekiti, Nigeria.
[3]Department of Chemical Sciences, College of Sciences, Afe Babalola University, Ado-Ekiti, Nigeria.
[4]Department of Environmental Health Sciences, Faculty of Public Health, University of Ibadan, Ibadan, Nigeria.

Enterococcus faecalis is a major cause of nosocomial infection in human and severe extra-intestinal infections in animals. *Enterococcus* spp possesses the ability to acquire and spread genes linked with antimicrobial resistance which makes them an important nosocomial pathogen. This study aims to determine the distribution of antibiotic resistance and virulence genotype of *Enterococcus faecalis* in animal, poultry and clinical sources. A total of 150 samples made up of poultry droppings, abattoir and clinical specimens were collected. Standard bacteriological methods were used in the isolation and characterisation of *E. faecalis.* Fifteen *E. faecalis* isolates were examined for virulence determinant genes through quantitative Polymerase Chain Reaction analysis, while disc diffusion technique was used in determining the antibiotic-resistant pattern of the isolates. The highest prevalence of *Enterococcus faecalis* was in poultry droppings (94.0%) and lowest in clinical samples (24.0%). Enterococcal surface protein *(Esp)* was detected in 2/7, 0/5 and 0/3 of poultry, abattoir and clinical isolates, respectively. Antibiotic susceptibility pattern of the isolates indicated high resistant in poultry, abattoir and clinical samples as follows: Erythromycin (87.2%), (100.0%), (100.0%); Cloxacillin (72.3%), (90.5%), (91.7%); Cefuroxime (68.1%), (100.0%), (66.7%) and Augmentin (63.8%), (81.0%), (66.7%). High prevalence of antibiotic resistance and high virulence potential were observed among the *E. faecalis* isolated. There is a need for constant epidemiological surveillance and strict enforcement of good hygiene practices in the study areas.

Key words: *Enterococcus faecalis,* antibiotic resistance, virulence, Nigeria.

INTRODUCTION

Enterococcus species have become one of the most common nosocomial pathogens, with patients having a high mortality rate of up to 61% (Shinde et al., 2012). They are of major importance in community-and hospital-

acquired infections, and estimated to be responsible for approximately 12% of all nosocomial infections globally (Shinde et al., 2012). *Enterococcus faecalis* has been reported to be the most predominant enterococcus species, accounting for 80 to 90% of all clinical isolates (Shinde et al., 2012).

Enterococci occur in a remarkable array of environments, since they are able to grow and survive under harsh conditions. They can be recovered from water, soil, food, and a variety of animals, birds and insects (Murray, 2010). In humans, the major habitat of enterococci appears to be the gastrointestinal tract although isolated less frequently from other body sites. *Enterococci* are contaminants of various foods; especially those of animal origin and are found as members of the natural microbiota of a variety of fermented food products such as artisanal cheeses and fermented sausages. They reportedly play an important role in food processing (Giraffa and Skinner, 2010; Franz et al., 2011). The transmission of resistant enterococci or resistance genes takes place between humans and animals in a community (Klare et al., 2005). One possible explanation for animal to human transmission was the use of glycopeptide growth promoters like Avoparcin in feed animals (Klare et al., 2005).

E. faecalis is the most commonly isolated species from root canal samples with treatment failure, periapical lesions or chronic apical periodontitis (Sedgley et al., 2013). Its present potential threat to general health is not yet known because endodontic infecting microorganisms are localized in a restricted area within the root canal system and little is known on the extra radicular presence of *E. faecalis* (Sunde et al., 2012). It is able to colonize the oral cavity, particularly in patients with periodontitis or root canal infections associated with oral mucosal lesions and in immunocompromised patients (Pinheiro et al., 2006). It is frequently isolated from patients suffering from dental infections like periodontitis, gingivitis, teeth with failed endodontics and infected root canals.

E. faecalis has some virulence determinants such as gelatinase (*gel*A), aggregation substance (*asa*1), cytolysin *cyl*A, enterococcal surface protein (*esp*) and collagen-binding protein (*ace*) and could be the reason for its survival in a harsh environment of the root canal system. *E. faecalis* adheres to host cells, express proteins that allow it to compete with other bacterial cells, and alter host responses. It is able to suppress the action of lymphocytes, potentially contributing to endodontic failure and also able to share these virulence traits among species, thus, further contributing to its survival and ability to cause diseases (Jett et al., 2014).

A contributing factor to the pathogenesis of *E. faecalis* is its evolving resistance to antibiotics. For instance, resistance to vancomycin is now widespread and common among members of the genus, which leaves few options for disease management (Rehaiem et al., 2014). There is a dramatic increase in antibiotic resistance of *Enterococcus* species worldwide highlighting the need for a greater understanding of this genus, including its ecology, epidemiology, and virulence (Ndubuisi et al., 2017).

However, little information is available on the virulence potentials of enterococci from different sources. There is a need to examine the occurrence of putative virulence factors by phenotypic and genotypic approaches in *E. faecalis* strains isolated from different sources such as abattoir, poultry and clinical origin in Ado-Ekiti, Ekiti State, Nigeria. More investigation on the potential virulence determinants and susceptibility patterns of different antibiotics would be useful in understanding their roles in disease causation. This study seeks to determine the frequency and distribution of antibiotic resistance and virulence genotypes of *Enterococcus faecalis* from poultry, animal and clinical sources.

MATERIALS AND METHODS

Collection of samples

One hundred and fifty samples were collected from three different sources, between August and November 2017. The sample collected was made up of poultry droppings (50), Abattoir (50), and Clinical specimens (50). Clinical specimens from wound infection, pus exudate, sputum and throat swab were collected from the Medical Bacteriology and Parasitology laboratory Department, Ekiti State University Teaching Hospital, Ado-Ekiti. Animal samples were collected from selected farms within Ado-Ekiti metropolis, including Afe Babalola University Farm, Ado-Ekiti, Nigeria and Elongated farms Ltd., Ado-Ekiti. Samples were collected in line with the procedure described by Diego et al. (2016).

Isolation and identification of *E. faecalis*

Microscopy and culture

Sample swabs were inoculated directly onto sterile plates of Bile esculin agar (Titan Biotech Ltd, Bhiwadi-301019, and Rajasthan, India). Swabs samples were processed within 6 h of collection, inoculated onto plates and incubated aerobically at 37°C for 24 h. The plates were examined for colonies with a characteristic dark reddish colour which is the presumptive feature for the identification of *Enterococcus* spp. on Bile esculin agar (Ekuma et al., 2016).

Discrete colonies of the isolates were picked from plates, subcultured onto MacConkey agar and incubated overnight at 37°C after which discrete colonies were picked and stored on nutrient agar slant at 4°C as stock. The isolates were identified using standard biotyping methods (Barrow et al., 2004; Fawole and Oso, 2001; Olutiola et al., 1991). Microbial surface colony count was carried out on the primary plate to determine the microbial load of each sample. Identification of *E. faecalis* was done using Gram stain and biochemical tests such as Catalase test, Motility test, and Esculin hydrolysis and heat resistance.

Antibiotics susceptibility testing

Susceptibility testing was carried out on Muller Hinton agar using disc diffusion method according to Clinical and Laboratory Standard Institute (CLSI, 2016; Yilema et al., 2017). The following antibiotics

disks (Abtek Biologicals, and Oxoid Ltd) with their concentration (in µg) which were previously identified from previous studies were used: Ceftazidine (30 µg), Cefuroxime (30 µg), Gentamycin (10 µg), Ceftriaxone (30 µg), Erythromycin (5 µg), Cloxacillin (5 µg), Ofloxacin (5 µg) and Augmentin (30 µg). A sterile wire loop was used: 3-5 well-isolated colonies were picked and mixed in nutrient broth. The prepared turbidity was matched with a turbidity standard (0.5 McFarland) to have an equal suspension.

A sterile swab was used to inoculate the suspension by streaking on the prepared and dried Muller Hinton agar plate evenly. Afterward, it was allowed to stay for 3-5 min. Sterile forceps were used to place the antimicrobial discs on the inoculated plates. Within 30 min after applying the disc, the plate was incubated at 35°C for 16-18 h. By using meter rule on the underside of the plate, diameter of each zone of inhibition was measured in millimetre (mm). Zone diameter for ATCC 25922, a recommended reference strain for antibiotic susceptibility testing was compared with National Committee for Clinical Laboratory Standards (NCCLS) published limits; interpretative charts were used to interpret the zone sizes of inhibition (Adesida et al., 2017). Results were recorded as susceptible or resistant based on the zones sizes of each of antimicrobial disc used (CLSI, 2016).

Molecular analysis

A total of 15 representatives of *E. faecalis* isolates made up of 7 from Poultry droppings, 5 from Abattoir and 3 from Clinical specimens respectively were selected for presence of virulence determinant genes that encodes the following genes; gelatinase (*gelA*), aggregation substance (*asa1*), Cytolysin *cylA*, enterococcal surface protein (*esp*) and Collagen-binding protein (*ace*) as well as confirmation of the bacteria identity using 16S-primer by polymerase chain reaction. The selection was based on their frequency of antibiotic resistance phenotypes.

Genomic DNA Extraction

DNA extraction was carried out according to the method of Thottappilly et al. (1999) and Olawale et al. (2015). A 60 g of the selected bacterial cells were suspended in 200 µl of water and added to ZR bashing beads lysis tubes: 800 µl of lysis buffer was added to the mixture. It was mixed briefly by vortexing and left to stand at room temperature for 5 min.

The mixture was transferred to a spin column in a collection tube and centrifuged at 10,000 rcf for one minute. The spin column was transferred to a new collection tube, 400 µl of DNA pre-wash buffer was added to the spin column and centrifuged at 10,000 rcf for one minute, 500 µl of g-DNA wash buffer was added to the spin column and centrifuged at 10,000 rcf for one minute. The spin column was transferred to a clean micro centrifuge tube; 100 µl DNA Elution buffer was added to the spin column, incubated for 2-5 min at room temperature and then centrifuged at top speed for 20 s to elute the DNA. The elute dDNA was stored at ≤-20°C for further use.

Molecular determination of *E. faecalis* virulence pattern by polymerase chain reaction and gel electrophoresis

Five designated bacteria virulence specific primer pairs (one forward and one reverse) were used on the genomic DNA of fifteen (15) bacterial isolates, one positive and negative control DNA samples. *E. faecalis* strains were first propagated using a modified procedure of Onasanya et al. (2003). A 200 µl amount of *E. faecalis* strain was transferred into 75 ml of nutrient broth in a 250 ml conical flask and kept under constant shaking at 37°C for 24 h. The bacterial cells were removed by centrifugation, washed with 0.1mM

Tris-EDTA and kept at -20°C for DNA extraction.

Genomic DNA extraction was performed according to Thottappilly et al. (1999) with some modification. Briefly, 0.3 g of washed bacterial cell was suspended in 200 µl of 2xCTAB buffer (50 mM Tris, pH 8.0; 0.7 mM NaCl; 10 mM EDTA; 2% hexadecyltrimethyl ammonium bromide; 0.1% 2-mercaptoethanol), followed by the addition of 100 µl of 20% sodium dodecyl sulfate (SDS) and incubated at 65°C for 20 min. DNA was purified by two extractions with an equal volume of chloroform and precipitated with 20°C absolute ethanol. After washing with 70% ethanol, the DNA was dried and re-suspended in 200 µl of sterile distilled water. DNA concentration was measured using DU-65UV spectrophotometer (Beckman Instruments Inc., Fullerton CA, USA) at 260 nm.

DNA degradation was checked by electrophoresis on a 1% agarose gel in 1 x TAE (45mM Tris-acetate, 1mM EDTA, pH 8.0). Quantitative Polymerase chain reaction (QPCR) was performed according to Creti et al. (2004) with some modifications. Briefly, oligonucleotide primers (Custom Primer Service, Life Technologies, and Gaithersburg, MD, USA) were used (Table 1). Two concentrations of each DNA preparation (24 and 96 ng per reaction) were used to test reproducibility and eliminate sporadic amplification products. Of the 6 primers used only three gave reproducible amplification products and were used, to amplify the DNA from all the 15 selected *E. faecalis* strains. Amplification was performed in 20 µl reaction mixture consisting of 4 µl genomic DNA, 2 µl 10X reaction buffer (Promega), 1 µl 25 mM dNTPs (dATP, dCTP, dGTP, and dTTP), 1 µl 2 mM primer, 1.6 µl 25 mM MgCl2 and 0.2 µl of 5 U/µl Taq polymerase (Boehringer, Germany), 2 µl 5% Tween-20, and 8.2 µl sterile distilled water.

The reaction mixture was overlaid with 50 µl of mineral oil to prevent evaporation. Amplification was performed in a thermowellmicrotiter plate (Costa Corporation) using a Perkin Elmer programmable Thermal Controller model 9600. The cycling program was 1 cycle of 94°C for 3 min followed by 32 cycles of 94°C for 20 s for denaturation, 54°C for 20 s for annealing of primer and 72°C for 40 s for extension; and a final extension at 72°C for 7 min. The amplification products were resolved by electrophoresis in a 1.4% agarose gel using TAE buffer (45 mMTris-acetate, 1 mM EDTA, pH 8.0) at 100 V for 2 h. A 1 kb ladder (Life Technologies, Gaithersburg, MD, USA) was included as a molecular size marker. Gels were visualized by staining with 0.5 g/ml ethidium bromide solution and banding patterns were photographed over UV light using a red filter.

RESULTS

The results of bacteria isolation and characterization showed 80(53.3%) yielded growth of *E. faecalis*, 70(46.7%) yielded growth of other organisms. Table 2 shows the distribution of *E. faecalis* isolated from various sources of samples studied, poultry droppings 47 (94.0%), abattoir 21 (42.0%) while clinical samples yielded the lowest growth of 12 (24.0%).

Multiple resistance patterns were obtained for *E. faecalis* isolated in poultry droppings (Figure 1). The highest resistance was obtained for antibiotics erythromycin (87.2%), while the lowest resistance was seen in ofloxacin with (44.7%). However, the isolates showed some level of susceptibility to ofloxacin (55.3%), augmentin (38.3%), ceftazidime (36.0%), ceferoxime (31.9%), cloxacillin (27.7%), ceftriaxone (23.4%), gentamycin (21.3%) and erythromycin (12.8%).

Table 1. PCR Primers selected for the detection of virulence genes in 15 samples of *E. faecalis* strains.

Target Gene	Sequence (5'- 3')	Position (bp)	Product Size (bp)
Esp	TTGCTAATGCTAGTCCACGACC	1217	932
	GCGTCAACACTTGCATTGCCGA	2149	
asal	CCAGCCAACTATGGCGGAATC	3122	529
	CCTGTCGCAAGATCGACTGTA	3651	
Ace	GGAATGACCGAGAACGATGGC	160	616
	GCTTGATGTTGGCCTGCTTCCG	776	

Table 2. Distribution of *E. faecalis* isolated from different sample sources.

Sample Source	Number collected	Number of *E. faecalis* isolates (%)	Other isolates (%)
Poultry	50	47 (94.0)	3 (6.0)
Abattoir	50	21 (42.0)	29 (58.0)
Clinical	50	12 (24.0)	38 (76.0)
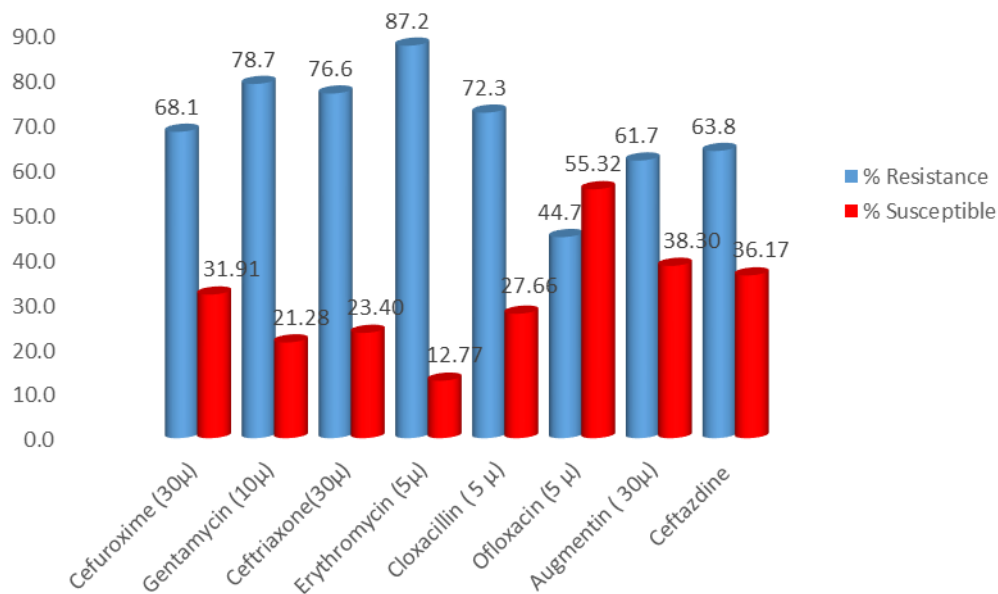
Figure 1. Antibiotics resistance pattern to poultry isolates.

The results of resistance pattern presented in Figure 2 shows antibiotics multiple resistance pattern with high frequencies to the following; augmentin (100.0%), erythromycin (100.0%), cefuroxime (100.0%), cloxacillin (90.5%) and ceftazidime (81.0%). Minimal susceptibility was seen in ofloxacin 14 (66.7%), gentamycin 13 (61.9%), ceftriaxone 9 (42.9%), ceftazidime (19.0) and lowest with cloxacillin (9.5%).

Multiple resistance patterns of *E. faecalis* isolates in clinical samples are indicated in Figure 3; the highest resistance to antibiotics used was Erythromycin, 12 (100.0%), Gentamycin, 11 (91.7%), Cloxacillin, 11 (91.7%), Ceftriaxone, 10 (83.3%), Augmentin, 8 (66.7%), and Cefuroxime, 8 (66.7%). Isolates equally showed some level of susceptibility to some antibiotics with highest seen in Ceftazdine, 8 (66.7%) and the lowest in
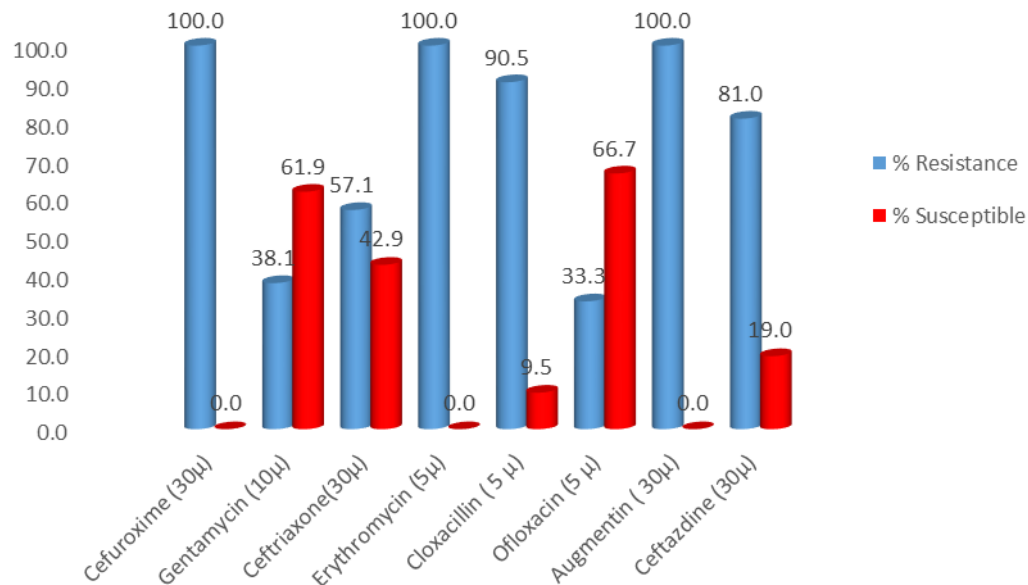
Figure 2. Antibiotics resistance pattern to abattoir isolates.
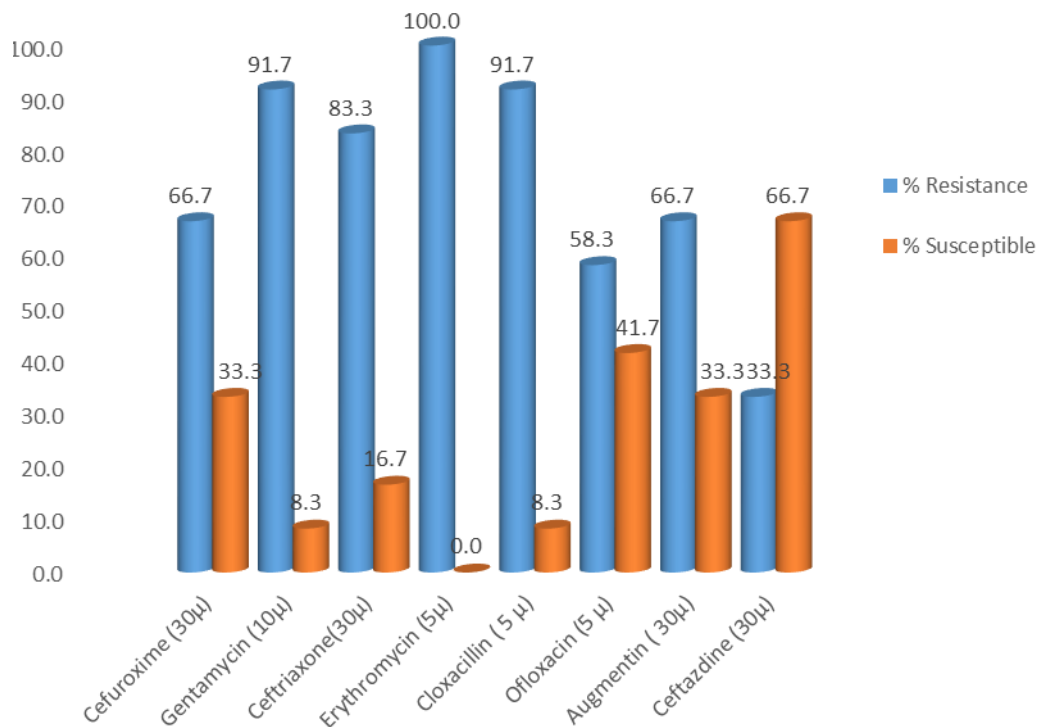
Figure 3. Antibiotics resistance pattern to clinical isolates.

Erythromycin 0 (0.0%).

In this present study, Table 3 indicates the frequency of resistance of antibiotics used against isolates of *E. faecalis* from various sources; poultry isolates exhibited high resistance to 7 out of 8 antibiotics used: erythromycin (87.2%), gentamycin (78.7%), ceftriaxone (76.6%),

cloxacillin (72.3%), cefuroxime (68.1%), ceftazidime (63.8%) and augmentin (61.7%). The pattern of resistance is also similar in abattoir isolates where 6 out of 8 antibiotics used show high resistance; erythromycin (100.0%), augmentin (100.0%), cefuroxime (100.0%), cloxacillin (90.5%), ceftazdine (81.0%) and ceftriaxone

Table 3. Multiple antibiotic resistance patterns to *Enterococcus faecalis* isolates from poultry droppings, abattoir, and clinical sources.

Samples	CRX	GEN	CTR	ERY	CXC	OFL	AUG	CAZ
Poultry	32 (68.1)	37 (78.7)	36 (76.6)	41 (87.2)	34 (72.3)	21 (44.7)	29 (61.7)	30 (63.8)
Abattoir	21 (100)	8 (38.1)	12 (57.1)	21 (100)	19 (90.5)	7 (33.3)	21 (100)	17 (81.0)
Clinical	8 (66.7)	11 (91.7)	10 (83.3)	12 (100)	11 (91.7)	7 (58.3)	8 (66.7)	4 (33.3)
Total	61 (76.3)	56 (72.5)	58 (72.5)	74 (92.4)	64 (80.0)	35 (43.8)	58 (72.5)	51 (63.8)

CRX , Cefuroxime (3 0µg); GEN , Gentamycin (10 µg); CTR – Ceftriaxone (30 µg); ERY , Erythromycin (5 µg); CXC , Cloxacillin (5 µg); OFL, Ofloxacin (5 µg); AUG, Augmentin (30 µg); CAZ, Ceftazdine (30 µg).

Table 4. Summary of distribution for virulence genes in *E. faecalis* isolate.

Source	Esp Gene	Asal Gene	Ace Gene
Poultry	2 (28.6%)	2 (28.6%)	2 (28.6%)
Clinical	0	0	0
Abattoir	0	3 (60.0%)	3 (60.0%)

Table 5. Distribution of Virulence Genes among selected isolates *E. faecalis* strains.

Serial number	Isolates	Sample source	*Esp* Gene	*Asal* Gene	*Ace* Gene
1	SP1A1	Poultry	-	-	-
2	SP1A2	Poultry	-	-	-
3	SP2B1	Poultry	-	+	-
4	SP3A1	Poultry	-	-	-
5	ABUAD-SPL32	Poultry	-	-	-
6	SP2A1	Poultry	+	+	+
7	PKL-41	Poultry	+	-	+
8	EKSG-3	Abattoir	-	+	+
9	EKSG-4	Abattoir	-	-	-
10	EKSG-7	Abattoir	-	-	+
11	EKSG-11	Abattoir	-	+	-
12	EKSG-20	Abattoir	-	+	+
13	EKUSTH-C18	Clinical	-	-	-
14	EKSUTH-C16	Clinical	-	-	-
15	EKSUTH-C3	Clinical	-	-	-
	Total		2	6	5
	(%)		13.3%	40.0%	33.3%

(57.0%).

The results obtained from Polymerase Chain Reaction (PCR) show the distribution of three genes virulence determinants: enterococcal surface protein (*esp*), aggregation substance *(asal)* and collagen-binding protein (*ace*) among the selected isolates. These findings are presented in Tables 4 and 5. The analyses show different prevalence of virulence genes in *E. faecalis* which range from 13.3 to 40.0%.

The PCR results showed that with P2 primer set, only isolates 2 (6 and 7) out of the fifteen isolates have *Esp* gene at specific PCR target 932 bp amplicon (Figure 4). Besides, there were other different PCR amplicons of varying molecular sizes indicating different *Esp* gene mutants. The primer P5 set revealed that *asal* gene at specific PCR target 529 bp amplicon was present in isolates 6 isolates (3, 6, 8, 9, 11 and 12) respectively (Figure 5). In addition, there were other different PCR amplicons of varying molecular sizes indicating the presence of different *asal* gene mutants. The performance
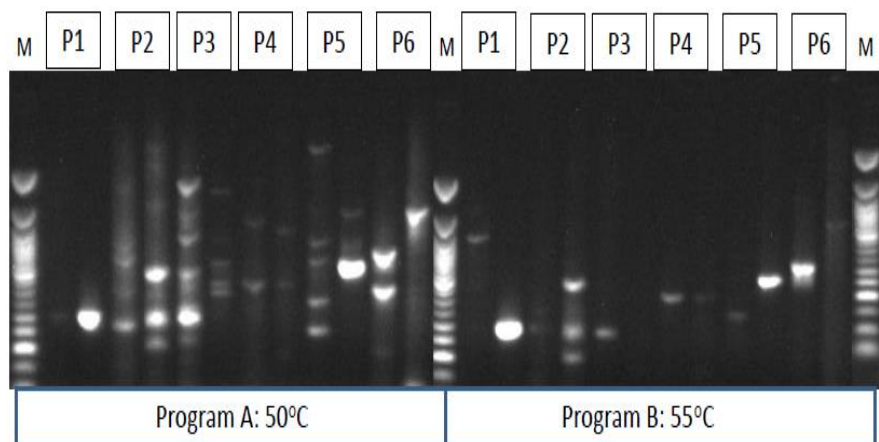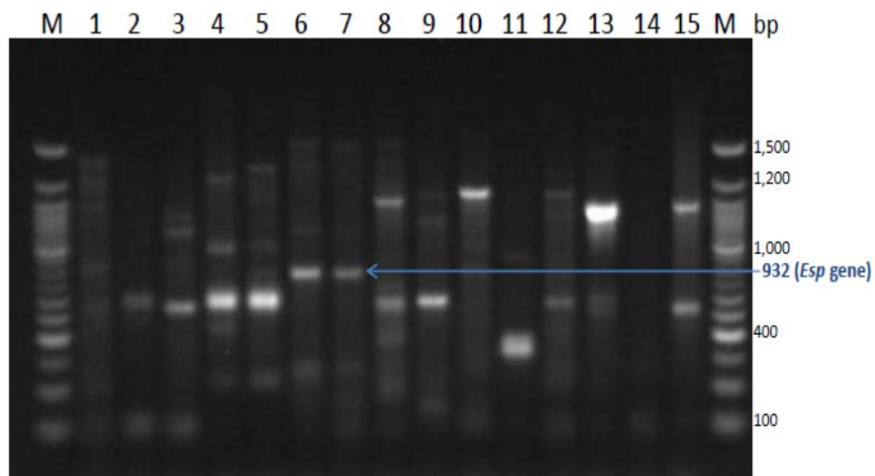
Figure 4. Results of Primer optimization.

Figure 5. PCR detection of virulence determinants *Esp* gene in *E. faecalis* strains as revealed by *Esp* specific primer. Lanes M Molecular marker, *E. faecalis* strains:1SPA1A;2 SPA1A2; 3SP2B1;4 SP3A1;5 ABUAD-SPL32;6 SP2A1;7 PKL-41;8 EKSG-3;9 EKSG-4;10 EKSG-7;11EKSG-11;12EKSG-20;13 EKSG-C18; 13 EKSUTH-C18;14 EKSUTH-C16;15 EKSUTH-C3

of primer P6 set showed the presence of *Ace* gene at the specific PCR target 616 bp amplicon in isolates (6, 7, 8, 10 and 12 respectively) (Figures 6 and 7). Also, there were other different PCR amplicons of varying molecular sizes which revealed the presence of different *ace* gene mutants.

DISCUSSION

E. faecalis are ubiquitous in their occurrence, with their habitats ranging from the intestinal tract of man and variety of farm animals to different forms of food and feed. In this present study, *E. faecalis* were isolated from poultry droppings, clinical specimens from pathological cases, and from abattoir products.

Findings from this study showed that the highest number of isolates of *E. faecalis* were from poultry dropping and least in clinical samples. The pattern of *E. faecalis* distribution in this study is in agreement with the result obtained from the study carried out by Hosseni et al. (2016), where *E. faecalis* was the most common species isolated from both dairy products and meat products. Virulence of *E. faecalis* was strongly enhanced
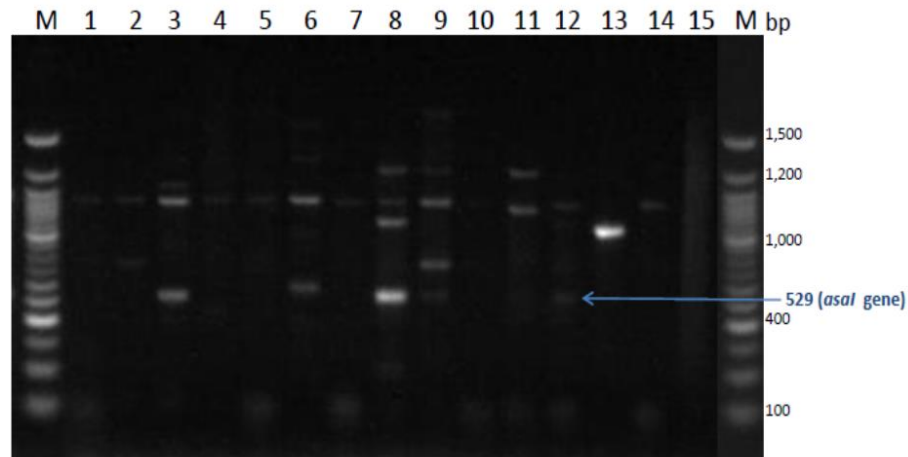
Figure 6. PCR detection of virulence determinants *asal* gene in *E. faecalis* strains as revealed by *asal* specific primer. Lanes M, Molecular marker; *E. faecalis* strains:1SPA1A; 2, SPA1A2; 3, SP2B1; 4, SP3A1; 5, ABUAD-SPL32; 6, SP2A1; 7, PKL-41;8,EKSG-3; 9, EKSG-4; 10, EKSG-7; 11, EKSG-11; 12, EKSG-20;13, EKSG-C18;13, EKSUTH-C18; 14, EKSUTH-C16; 15, EKSUTH-C3.
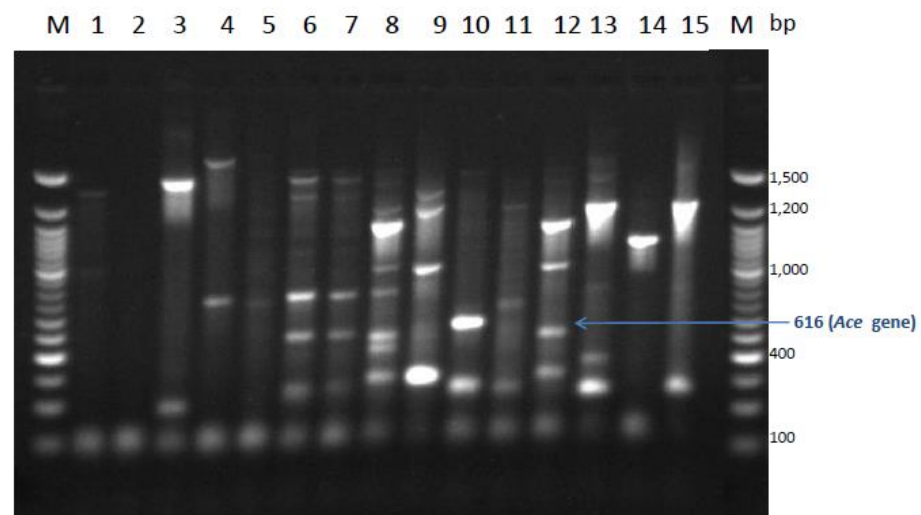
Figure 7. PCR detection of virulence determinants *ace* gene in *E. faecalis* strains as revealed by *ace* specific primer. Lanes M, Molecular marker; *E. faecalis* strains:1SPA1A; 2, SPA1A2; 3, SP2B1; 4, SP3A1; 5, ABUAD-SPL32; 6, SP2A1; 7, PKL-41;8,EKSG-3; 9, EKSG-4; 10, EKSG-7; 11, EKSG-11; 12, EKSG-20;13, EKSG-C18;13, EKSUTH-C18; 14, EKSUTH-C16; 15, EKSUTH-C3.

by their frequent resistance to commonly used antibiotics. Antibiotics which can be both intrinsic and acquired make *E. faecalis* effective opportunists in nosocomial infections. In this study, poultry isolates exhibited high resistance to Erythromycin, Gentamycin, Ceftriaxone, Cloxacillin, Cefuroxime, Ceftazidime, and Augmentin. The pattern of resistance was also similar in abattoir isolates where 6 out of 8 antibiotics used showed high resistance. These findings are in conformity with that of Trivechi et al. (2011) where high resistance pattern to erythromycin, ofloxacin, and cephalothin was reported in *E. faecalis* isolated from dairy and poultry products. Olawale et al.

8

(2015), also reported high resistance pattern for erythromycin, cloxacillin, augmentin, and gentamycin.

E. faecalis isolates from clinical samples in this study showed high resistance to most antibiotics used. The intrinsic resistance to many available antibiotics suggests that the treatment of an infection with *E. faecalis* could be difficult. Also, the major risk- associated with this resistance is that they are transferable, the gene coding for all of these antibiotic-resistant traits may be transferred by pheromone-mediated, conjugative, plasmids or transposons. However, emergence of resistance to common antibiotics in these isolates has compromised the clinical usefulness of several antimicrobial classes, including macrolides, amino-glycosides, and glycopeptides. The need for sourcing for other antimicrobial agents is important and critical for continued access to antimicrobials with clinical efficacy and balance of potencies against *E. faecalis* emerging pathogens (Oranusi et al., 2013).

The results obtained from PCR analysis showed the distribution of three genes virulence determinants: enterococcal surface protein (*esp*), aggregation substance *(asal*) and collagen-binding protein (*ace*) among the selected isolates. Virulence determinant, aggregation substance, and collagen-binding protein were common to isolates from poultry and abattoir. This revealed that there was a high distribution of virulence potentials among *E. faecalis* from poultry and abattoir origin.

It has been reported that *ace* genes are very important virulence-associated factors (Singh et al., 2010). However, finding in this study was able to detect *ace* and *asal* genes in both poultry and abattoir strains; none of these genes should be considered definitive markers of pathogenicity in poultry *E. faecalis;* it is more likely they contribute to virulence potential of *E. faecalis*, but is independent of additional virulence factor present or a decreased disease-resistance of the host before causing infection (Marra et al., 2007).

Aggregation substance gene (asal) was also common among the poultry and abattoir strains, but not detected in clinical strains. Previous studies of the occurrence of *asal* in enterococcal isolates are contradictory; some indicated a higher prevalence of *asal* in clinical isolates compared to other sources (Waar et al., 2002), whereas other studies found a similar prevalence (Huycke and Gilmore, 1995).

The gene encoding the enterococcal surface protein, (*esp)*, has been associated with increased virulence, colonization and biofilm formation (Rahmadan and Hegedus, 2005; Latasa et al., 2006). The *esp* was the least frequently detected virulence gene in poultry isolates. This is in agreement with previous reports from studies carried out by Hammerum and Jensen (2002) and Poeta et al. (2006).

Conclusion

E. faecalis are widespread in the environment and have emerged as opportunistic pathogens, responsible for increasing the percentage of nosocomial infections. High prevalence of virulence potentials among *E. faecalis* from poultry and abattoir origin were determined in this study. The high degree of contamination of animal source analysed was, therefore, an indicator of how high the probability of colonization by these microorganisms of human intestine can be. *E. faecalis*, however, should be considered not only as potential pathogens but also as a reservoir of genes encoding for antibiotic resistance that can be transferred to other pathogenic and non-pathogenic microorganisms. Distribution of potentially virulent *E. faecalis* in this study area portends danger for reservoir of high antibiotic resistance pathogens and serious health hazards. This should necessitate adoption of stringent infection control measures. There is a need for improved hygiene practices with strict enforcement of good manufacturing practices in the study location and beyond. Further investigations such as phylogenetic analysis are required to evaluate the similarity of virulence genes on nucleotide level between poultry, abattoir and clinical isolates.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Adesida SA, Ezenta CC, Adagbada AO, Aladesokan AA, Coker AO (2017). Carriage of Multidrug Resistant Enterococcus faecium and Enterococcus Faecalis among apparently Healthy Humans. African Journal of Infectious Disease 11(2):83-89.

Barrow GI, Feltham RK, Cowan A, Stell S (2004). Manual for Identification of Medical Bacteria. Cambridge University Press 331 p.

CLSI (2016). Performance Standard for Antimicrobial Disk Susceptibility: Tests approved standard, Eleventh Edition, M02-A11-32c11.Replaces M02-A10:2a (1).

Creti R, Imperi M, Bertuccini L, Fabretti F, Orefici G, Di Rosa R (2004). Survey for virulence determinants among *Enterococcus faecalis* isolated from different sources. Journal of Medical Microbiology 53:13-20.

Diego C, Andrighetto C, Lombardi A (2016). Presence of virulence factor and antibiotics resistance in *Enterococcous* spp collected from dairy products and meat. Food Control 19(9):886-892.

Ekuma AE, Oduyebo OO, Efunshile AM, Konig B (2016). Surveillance for Vancomycin Resistant Enterococci in a Tertiary Institution in South Western Nigeria. African Journal of Infectious Disease 10(2):121-126.

Fawole MO, Oso BA (2001). Laboratory Manual of Microbiology, Spectrum Books Ltd, Ibadan 127 p.

Franz SK, Edwards JR, Courval JM, Hill H, Tenover FC, Lawton R (2011). Intensive Care Antimicrobial Resistance Epidemiology (ICARE) Project and the National Nosocomial Infections Surveillance (NNIS) System Hospitals. The effect of vancomycin and third-generation cephalosporins on prevalence of vancomycin-resistant

enterococci in 126 U.S. adult intensive care units. Annal of Internal Medicine 135:175-183.

Giraffa MF, Skinner FD (2010). Origin, evolution and phylogeny of giraffes camelopardis. Transctions of Royal Society of South Africa 58:234-241.

Hammerum AM, Jensen LB (2002). Prevalence of *Esp,* encoding the enterococcal surface protein in *E. faecalis* and *E. faecium* isolates from hospital patients, poultry, and pigs in Demark. Journal of Clinical Microbiology 40:43-96.

Hosseni SM, Zeni B, Ratyani S, Jafari R, Shambo F, Tabar ZK, Arabestani MR (2016). Presence of virulence factors and antibiotics resistance in *Enterococcus* spp collected from dairy product and meat. Der Pharmacia letters 8(4):138-145.

Huycke MM, Gilmore MS (1995). Frequency of aggregation substance and cytolysin genes among enterococcal endocarditis isolates. Plasmid 34:152-156.

Jett BD, Huycke MM, Gilmore MS (2014). Virulence of enterococci. Clinical Microbiology Review 7:462-478.

Klare I, Heier H, Claus H, Bohrne *G,* Martins M, Seltmann G (2005). *Enterococcus faecium* strains with vanA-mediated high-level glycopeptide resistance isolated from animal foodstuffs and faecal samples of humans in the community. Microbial Drug Resistant pp. 265-272.

Latasa CC, Solano JR, Penades Lasa I (2006). Biofilm associated proteins. C.R. Biology 329:849-857.

Marra AF, Dib-Hajj I, Lamb F, Kaczmarek WC, Shang G, Beckius AJ, Milici I, Medina Gootz TD (2007). Enterococcal virulence determinants may be involved in resistance to clinical therapy. Diagnostic Microbiology Infection Diseases 58:59-65.

Murray BE (2010).The life and times of the *Entcrococcus.* Clinical Microbial Reviews 3:46-65.

Ndubuisi JC, Olonitola OS, Olayinka, AT, Jatau ED, Iregbu KC (2017). Prevalence and antibiotics susceptibility profile of *Enterococcus* spp isolated from some hospitals in Abuja, Nigeria. African Journal of Clinical and Experimental Microbiology 18(3):154-158.

Olawale AK, Salako RJ, Olawale AO, Famurewa O (2015). Antibiotics-Resistant *Enterococcus Faecalis* isolated from canteens in Osun State, Nigeria. British Microbiology Research Journal 6(4):196-206.

Olutiola PO, Famurewa O, Sonntag HC (1991). Introduction to General Microbiology: A practical approach. Heidelberger veriagasanstalt and Druckerei, GmbH, Heidelberg.

Onasanya A, Mignouna HD, Thottappilyy G (2003). Genetic finger printing and phylogenetic diversity of isolates of *Staphylococcus aureus* from Nigeria. African Journal of Biotechnology 2:246-250.

Oranusi S, Onyeike E, Galadima M, Umoh VJ (2013). Hazard analysis critical control points of food prepared by families in Zaria, Nigeria. Nigeria Journal of Microbiology 18(1-2):346-362.

Pinheiro ET, Anderson MJ, Gomes BPFA, Drucker DB (2006). Phenotypic and genotypic identification of enterococci isolated from canals of root-filled teeth with periapical lesions. Oral Microbiology Immunology 21:137-144.

Poeta P, Costa D, Rodrigues J, Torres C (2006). Detection of genes encoding virulence factors and bacteriocins in fecal enterococci of poultry in Portugal. Avian Diseases 50:64-68.

Rahmadan AA, Hegedus E (2005). Biofilm formation and *esp* gene carriage in enterococci. Journal of Clinical Pathology 58:685-686.

Rehaiem A, Belgacem ZB, Edalatian MR, Martínez B, Rodríguez A, Manai M, Guerra NR (2014). Assessment of potential probiotic properties and multiple bacteriocin encoding-genes of the technological performing strain *Enterococcus faecium* MMRA. Food Content 37:343-350.

Sedgley C, Nagel A, Dahlen G, Reit C, Molander A (2013). Real-time quantitative polymerase chain reaction and culture analyses of *Enterococcus faecalis* in root canals. Journal of Endodology 32:173-177.

Shinde RS, Koppikar GV, Oommen S (2012). Characterization and antimicrobial susceptibility pattern of clinical isolates of Enterococci at a tertiary care hospital in Mumbai, India. Annal of Tropical Public Health 5(2):85-88.

Singh KV, Nallapardy SR, Sillanpaa J, Murray BE (2010). Importance of the collagen adhesion Ace in pathogenesis and protection against *E. faecalis* experimental endocarditis. Plos Pathologens 6(1):323-331.

Sunde PT, Olsen I, Debelian GJ, Tronstad L (2012). Microbiota of periapical lesions refractory to endodontic therapy. Journal of Endodology 28:304-310.

Trivechi K, Cupakova S, Karpiskova R (2011). Virulence factors and antibiotics resistance in enterococcus isolated from food-stuffs. Veterinarian Medicina 56(7):352-357.

Thottappilly G, Mignouna HD, Onasanya A, Abang M, Oyelakin O, Singh NK (1999). Identification and differentiations of isolates of *Collectotrichum gleosporioides* from yam by random amplified polymorphic DAN makers. African Crop Science Journal 7(2):7213-7218.

Waar K, Muscholl-Silberhorn AB, Williams RJI, Slooff HJM, Dengner JE (2002). Geno grouping and incidence of virulence factors of *enterococcus faecalis* in liver transplant patients differ from blood culture and fecal isolates. Journal of Infectious Diseases 185:1121-1127.

Yilema A, Moges F, Tadele S, Endris M, Kassu A, Abebe W, Ayalew G (2017). Isolation of enterococci, their antimicrobial susceptibility patterns and associated factors among patients attending at the University of Gondar Teaching Hospital. BMC Infectious Diseases 17:276.

African Journal of Microbiology Research

Full Length Research Paper

Evaluation of three commercialized rapid point-of-care tests for detection of anti-hepatitis C virus antibodies in Burkina Faso

Ouedraogo H. Gautier[1,2]*, Ouandaogo H. Siaka[2], Zida Sylvie[1], Compaoré T. Rebeca[1], Sagna Tani[1], Sawadogo Salam[3], Kouanda Seni[1], Traoré Yves[2] and Barro Nicolas[2]

[1]Laboratoire de Recherche Biomédicale, Département Biomédical et Santé Publique, Institut de Recherche en Sciences de la Santé (IRSS), Ouagadougou, Burkina Faso.
[2]Departement de Biochimie-microbiologie, Centre de Recherche en Sciences Biologiques Alimentaires Nutritionnelles (CRSBAN), Université de Ouagadougou, Burkina Faso.
[3]Centre Régional de Transfusion Sanguine (CRTS), Ouagadougou, Burkina Faso.

This study aimed to evaluate the diagnostic performance of commercially rapid point-of-care (POC) tests used for HCV antibodies detection. This is a case-control study conducted in Ouagadougou between December 2014 and January 2015. Three POC for HCV antibodies detection (*SD Bioline HCV test®, Anti-HCV dipstick® and First response® HCV card test*) marketed in Burkina Faso were evaluated. Architect anti-HCV assay and ImmunoComb® II HCV were combined and used as a reference test. All three tests were evaluated with a panel of 62 anti-HCV positive sera and 62 anti-HCV negative sera. The tests performance was calculated using the software OpenEpi. The three rapid POC tests had a specificity of 100% (95% CI: 94.17-100). However, the sensitivities were 33.87% (95% CI: 23.34-46.28) for the *SD Bioline HCV test®*, 41.94% (95% CI: 30.48-54.33%) for *Anti-HCV dipstick®* and 45.16% (95%CI: 33.42-57.47%) for *First response® HCV card test*. The tests evaluated in this study had good specificity but poor sensitivity for the HCV antibodies detection in Burkina Faso. The surveillance of HCV rapid POC tests through the validation of their accuracy in the local context before their approval must be strengthened.

Key words: Hepatitis C virus, point-of-care, diagnostic test.

INTRODUCTION

Hepatitis C virus (HCV) is a hepatotropic RNA virus of the *Hepacivirus* in the Flaviviridae family (Chevaliez and Pawlotsky, 2006). It is responsible for both acute and chronic liver infection. According to the World Health Organization (WHO), more than 71 million people are infected worldwide (WHO, 2019), and new infections

were estimated at 1.75 million in 2015 (WHO, 2017). Transmission of HCV is mainly through blood (blood transfusion, injection with non-sterile needles, tattoos and other traditional practices, etc.), but it can also be transmitted through sexual intercourse or by an infected mother to her infant (Moosavy et al., 2017). While some infected people may spontaneously clear the virus, the infection progresses to chronicity in 60 to 80% of cases with the appearance of severe liver injury (cirrhosis, and hepatocellular carcinoma) in the long term (Moosavy et al., 2017; Spearman et al., 2019).

The prevalence of HCV remains highly variable from one region to another in Sub-Saharan Africa. Indeed, in a systematic review, the authors estimated HCV seroprevalence in general population in East and South Africa at 0.91% (95% CI: <0.80-1.20), 4.34% (95% CI: 3.99- 4.70) in West Africa and 6.76% (95% CI: 5.98-7.55) in Central Africa (Semugoma et al., 2017). In Burkina Faso, hepatitis C testing on the blood samples collected during the 2010 Demographic and Health Survey (DHS) estimated HCV prevalence at 3.5% (95% CI: 3.0-3.9) (Madiou, 2016). In blood donors, studies have estimated the prevalence of HCV at 8.69% at the Regional Center for Blood Transfusion (CRTS) of Koudougou (Nagalo et al., 2011), and at 4.4% at the CRTS of Ouagadougou (Zeba et al., 2014). This high prevalence of HCV in blood donors justifies the inclusion of screening of this virus in the routine testing of blood donation in the country's blood banks, in addition to HIV, hepatitis B virus and syphilis. The screening for HCV in the blood donation is done using Enzym Linked Immuno Sorbent Assay (ELISA). However, these ELISA technics require relatively expensive laboratory equipment, reagents and relatively long handling times. In the context of frequent blood shortages and emergencies due to traffic accidents and surgical or obstetric complications, rapid HCV tests is often used as an emergency for HCV testing in some blood banks. These tests are very easy to use, provide results in less than 30 min and are less expensive, especially in resources limited contexts (Drain et al., 2014). They do not require highly qualified laboratory technicians, or specific laboratory equipment, or even electricity and cold chain storage. However, even if the manufacturers of these tests reported that they have a very good diagnostic performance (high sensitivity and specificity), the quality and reliability of some of these tests are often questioned (Drain et al., 2014; Khuroo et al., 2015; O'Connell et al., 2013; Scheiblauer et al., 2006; Tang et al., 2017).

Several rapid screening tests from various sources and brands are marketed and used for HCV antibody screening in Burkina Faso, but there are no data comparing these rapid HCV tests with routine ELISA methods practiced in the country's blood banks. This study aimed to assess their diagnostic performance in the screening of HCV antibodies in Burkina Faso. If these tests are efficient, they can be used as an alternative in laboratories in limited resources contexts.

METHODOLOGY

Study design

This is a cross sectional study conducted in Ouagadougou. The evaluation included three (3) point-of-care (POC) tests marketed in Burkina Faso for qualitative detection of hepatitis C virus antibodies.

Rapid POC tests evaluated

Three (3) anti-HCV rapid POC tests marketed in Burkina Faso were evaluated. The POC tests included the following: *SD Bioline HCV test®*, *First response® HCV card test* and *Anti-HCV dipstick test®*. All tests were obtained free of charge from the suppliers for the evaluation. The characteristics of the POC tests are presented in Table 1.

Samples collection and laboratory methods

Samples were collected between December 2014 and January 2015 in the Regional Blood Transfusion Center (CRTS) of Ouagadougou, where all blood donations are tested serologically for HIV, hepatitis B, C and syphilis. After consent was obtained from the donor, 10 mL of blood were taken from the tubing of the blood collection bag in two dry tubes 5 mL Vacutainer®, then kept in the refrigerator at + 4 ±2°C while waiting for the results of screening for syphilis, HIV, hepatitis B and C viruses as part of the blood donation.

Once, the infection status of blood donations established through the initial analyzes performed by the CRTS (ELISA test HCV, HBV, HIV and syphilis), the positive samples for anti-HCV and those negative for anti-HCV antibodies were preselected. All reference samples were negative for HIV, syphilis and HBV. The anti-HCV screening as part of the qualification of blood donation at the CRTS was done using the Architect Anti-HCV® automatized assay (Abbott Diagnostics). Architect Anti-HCV assay is an automated test designed for chemiluminescent immunoassay (CMIA) immunoassay for the qualitative detection of antibodies to hepatitis C virus (HCV) in human serum and plasma. Its specificity according to the manufacturer was evaluated at 99.6% (95% CI: 99.45-99.71) and its sensitivity is 99.10% (95% CI: 96.77-99.99) (ABBOTT Diagnostics Division, 2009).

The preselected samples were centrifuged at 3000 rpm for 10 min, and then the serum was put in two 2 mL cryotubes, labeled and stored at -20°C. In order to confirm the presence or absence of anti-HCV in these samples, they were re-tested using the ImmunoComb® II HCV test (Orgenic Ltd). This second testing was performed to avoid inclusion of false positive or false negative samples in the panel, due to the high sensitivity of the Architect Anti-HCV test. The ImmunoComb® II HCV Assay is a sensitive and specific assay for the detection of anti-HCV antibodies. These diagnostic performances have been confirmed in earlier studies in Africa compared to other tests (Njouom et al., 2006)

At the end of this second testing, 62 anti-HCV positive samples and 62 anti-HCV negative samples served as a reference panel for the evaluation.

All rapid POC were evaluated using the panel of samples in accordance with manufacturers' requirements and Good Laboratory

Table 1. Characteristics of the evaluated rapid POC tests according to the manufacturers.

Characteristics	SD Bioline HCV test®	First response® HCV card test	Anti-HCV dipstick®
Manufacturer	Standard Diagnostics, Inc.	Premier Medical Corporation Ltd	CYPRESS Diagnostics
Principle of the test	immunochromatography	immunochromatography	immunochromatography
Antigen	(Core, NS3, NS4, NS5)	(Core, NS3, NS4, NS5).	--
Product code	02FK11	I03FRC	172-050/S
Batch number	02BM14013	39K0214D	B201503056
Expiration date	2016/07/07	2016/05	2017/03
Biological sample	Serum or plasma	Serum, plasma or whole blood	Serum or plasma
Storage	1-30°C	4-30°C	10-30°C
Reading	Visual	Visual	Visual
Time for result	15 min	15 min	15 min
Reading time (stable result)	20 min	20 min	20 min
Consumable required not provided	Pipette	-	-
Sensitivity according to the manufacturer	100%	99.75%	98.7%
Specificity according to the manufacturer	99.4%	99.5%	95.6%

Table 2. Results of the evaluated rapid POC tests on the panel of serum samples.

POC tests	Result	Architect Anti-HCV+ImmunoComb® II HCV	
		Positive (n=62)	Negative (n=62)
SD Bioline HCV test®	Positive	21 TP	00 FP
	Negative	41 FN	62 TN
Total		62	62
First response® HCV card test	Positive	28 TP	00 FP
	Negative	34 FN	62 TN
Total		62	62
Anti-HCV dipstick®	Positive	26 TP	00 FP
	Negative	36 FN	62 TN
Total		62	62

TP, True positive; FP, false positive; TN, True negative; FN, false negative.

Practices (GLP). In order to avoid comparing the results between the tests during the laboratory analysis, each rapid assessment test was tested in a series with all the panel samples before proceeding to another test. The results interpretation was blind, regardless of the results of the reference test.

Data processing and analysis

The test results were entered into Excel 2013 file. For each sample tested, the test results were compared to the positive or negative status of the reference test and categorized as true positive, false positive, true negative and false negative. The sensitivity and specificity of the rapid POC in evaluation were then calculated using free and open source epidemiological statistics software, OpenEpi (http://www.openepi.com,). Cohen's Kappa coefficient (Cohen, 1960) was used to assess the concordance of rapid POC tests with the reference test. Interpretation of the Kappa results was made according to the following criteria: a value ≤ 0 indicating "the absence of agreement" and 0.01-0.20 as a "very weak agreement",

0.21-0.40 as "weak agreement", 0.41-0.60 as "moderate agreement", 0.61-0.80 as "strong agreement" and 0.81-1.00 as "almost perfect agreement"(McHugh, 2012).

RESULTS

The results obtained after the analysis of the 62 anti-HCV positive samples and the 62 anti-HCV negative samples with the index tests are recorded in Table 2. None of the three tests yielded a false positive. In contrast, all three tests each yielded more than 50% false anti-HCV negative.

The evaluated tests diagnostic performance is shown in Table 3. All three rapid POC tests had a specificity of 100% (95% CI: 94.17-100).The sensitivities of these tests were different and varied between 33.87% (95% CI: 23.34-46.28) for the "SD Bioline HCV test®" and 45.16%

Table 3. Diagnostic performance of the tests according to the evaluation.

Parameter	Anti-HCV dipstick		First response® HCV card test		SD BIOLINE HCV test	
	Estimation	95%CI	Estimation	95%CI	Estimation	95%CI
Sensitivity	41.94%	30.48 - 54.33	45.16%	33.42 - 57.47	33.87%	23.34 - 46.28
Specificity	100%	94.17 - 100	100%	94.17 - 100	100%	94.17 - 100
Diagnosis accuracy	70.97%	62.44 - 78.23	72.58%	64.14 - 79.67	66.94%	58.26 - 74.6
Youden (J)	0. 4194	0.2465 - 0.5433	0.4516	0.2759 - 0.5747	0.3387	0.1751 - 0.4628
Efficiency	0.7096		0.7258		0.6693	
Cohen Kappa coefficient	0.4194	(0.2761 - 0.5627)	0.4516	(0.3044 - 0.5988)	0.3387	(0.2067 - 0.4707)

(95% CI: 33.42-57.47) for the Anti-HCV dipstick Kit. Similarly, the Cohen's kappa concordance with the reference test are respectively 0.3387 (95% CI: 0.1751-0.4628) for the *"SD Bioline HCV test®"*; 0. 4194 (95% CI: 0.2465-0.5433) for the *"Anti-HCV dipstick®"* and 0.4516 (95% CI: 0.2759-0.5747) for *"First response® HCV card test"*.

The study shows a rather simple use of these three (3) tests. However Anti-HCV dipstick is even simpler than the other two because of its strip structure and the absence of migration diluent. In terms of the equipment needed to perform the test, but not provided, only the *"SD Bioline HCV test®"* did not have pipettes in its kit. The time required to obtain the results is identical for the three tests (20 min). The interpretation of the results is very easy, and visual for all three (3) tests.

DISCUSSION

This study shows that the three rapid HCV tests evaluated have very good specificity (100%), but low sensitivity in the detection of HCV antibodies. These tests have low to moderate concordance with the reference test (McHugh, 2012). With a sensitivity of 45.17%, *First response® HCV card test* was the most sensitive of the three tests evaluated. It is followed by *Anti-HCV dipstick®* (41.74%) and then by *SD Bioline HCV test®* (33.87%). This means that each of these three tests yields more than 50% of false negatives in subjects with HCV. If used in blood transfusion, many blood bags would fail to be sensitive, putting the health of recipients at risk.

In this study, all the false results are false negatives, hence a very good specificity observed for the rapid POC evaluated. Most studies confirm the high specificity of rapid tests for anti-HCV (Tang et al., 2017). However, because of their low sensitivity, the diagnostic accuracy of some of them is often poor. The most likely assumption underlying the low sensitivity of the evaluated rapid POC tests for detection of HCV antibodies, is that these tests have a high detection limit compared to the reference test. A study comparing Rapid POC tests with EIA methods had already shown that the sensitivity of rapid

HCV antibodies tests decreased with the concentration of anti-HCV in the blood (Montebugnoli et al., 1999). However, the sensitivities found in this study are below those indicated by the tests manufacturers. Indeed, the sensitivities of these three rapid tests according to the manufacturer's instructions are between 95.29 and 100%, and their specificities are between 98.75 and 99.5%. These standards are based on different studies generally conducted under different conditions than those of resource-limited countries in terms of quality insurance. WHO (World Health Organization) has clearly demonstrated the high probability of obtaining false results by using Rapid POC tests in the event of non-compliance with quality assurance (Adler et al., 2015). Nevertheless, the results we obtained from these rapid POC tests suggest that further studies be conducted to establish an algorithm using these rapid POC tests for the detection of HCV infection in Burkina Faso. The literature reports little or no data on the performance of *Anti-HCV Dipstick®* and the *First Response HCV Card test®*, as opposed to the *SD Bioline HCV test®*. In a systematic review and meta-analysis conducted by Mehnaaz et al. (2015), the sensitivity (pooled sensitivity) of SD Bioline HCV was estimated 93.5% with intervals of 73.2 to 98.7%. Which is high compared to the sensitivity found in this study (Khuroo et al., 2015).

However, this study is not the first to report low sensitivity for rapid HCV testing. Mehnaaz et al. (2015) found that the anti-HCV rapid POC tests had good overall accuracy, but this accuracy was very heterogeneous between the individual tests. According to these authors, the sensitivity and specificity of these tests ranged from 16.0 to 99.9% and from 77.8 to 99.7%, respectively (Khuroo et al., 2015). Another study found that the rapid tests evaluated had a sensitivity of 34.5% (95% CI, 25.0-45.1%) for CORE HCV® (CORE Diagnostics, Birmingham B2 5HG, United Kingdom) and 98.8% (95% CI, 94.3-99.9%) for OraQuick HCV® (Meridian Bioscience, Inc. London). However for these tests the specificities were all very high (O'Connell et al., 2013). Added to this are the sensitivities of 49% for the ACON HCV® test (ACON Laboratories, San Diego, CA, USA), 63.1% for the Labmen TM test (Chevaliez et al., 2016a), 64% for the

Hexagon HCV® test (Human Gesellschaft für Biochemica und Diagnostica, Wiesbaden, Germany) in Cameroon (Njouom et al., 2006).

The high variability in the performance of hepatitis C virus rapid POC tests calls for great caution in the selection and use of these tests. Choosing a poorly performing test for HCV screening could have adverse public health consequences, especially when they provide false negative results. Used in a blood bank for the screening of donated blood, these tests of low analytical sensitivity can generate a serious threat to the recipients (Khuroo et al., 2015; Pruett et al., 2015). The sensitivity deficiency of the tests evaluated in this study recommends a prior and serious assessment of HCV Rapid POC tests performance prior to their registration and marketing in resource-limited settings. However, the results should be considered with some contextual limitations. Firstly, there were no conditions of traceability and conservation of these tests were provided free of charge by local suppliers. Although this limit is valid, the results report the performance of routine tests commonly used in laboratories and hospitals across the country, as provided by local distributors. Secondly, it is recognized that HCV has a high genetic diversity with six known genotypes (Chevaliez and Pawlotsky, 2006; Zeba et al., 2014). This genetic diversity could have a significant effect on the diagnostic accuracy of marketed tests. The study did not take into account HCV genetic diversity, but studies are contradictory as to this influence (Khuroo et al., 2015; Scheiblauer et al., 2006; Tang et al., 2017).

In conclusion, the rapid HCV test samples evaluated in this study are poor at detecting anti-HCV. These results challenge the various actors involved in the HCV prevention chain, including health system decision-makers, hospitals, laboratories and reagent providers, to be more vigilant in choosing Rapid POC tests for screening for this virus in Burkina Faso. The high cost and complexity of ELISA and molecular biology methods in the context of limited resources require alternative methods such as Rapid POC tests. However, these Rapid POC tests must be of high diagnostic performance to play their part in HCV testing. It is important that health authorities strengthen the surveillance of HCV rapid diagnostic tests marketed in Burkina Faso, by validating their performance in the local context before their approval.

CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

REFERENCES

ABBOTT Diagnostics Division (2009). ARCHITECT Anti-HCV.

Adler M, Behel S, Duncan D, Houston J, Kalou M, Lasry A, Shaffer N, Sands A, Young PR (2015). Technical guidance update on quality assurance for HIV rapid diagnostic tests. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK316036/

Chevaliez S, Poiteau L, Rosa I, Soulier A, Roudot-Thoraval F, Laperche S, Hézode C, Pawlotsky JM (2016a). Prospective assessment of rapid diagnostic tests for the detection of antibodies to hepatitis C virus, a tool for improving access to care. Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases 22(5):459.e1-6.

Chevaliez S, Pawlotsky JM (2006). HCV Genome and Life Cycle. In: S.-L. Tan (Ed.), Hepatitis C Viruses: Genomes and Molecular Biology. Retrieved from http://www.ncbi.nlm.nih.gov/books/NBK1630/

Cohen J (1960). A coefficient of agreement for nominal scales. Educational and Psychological Measurement 20(1):37-46.

Drain PK, Hyle EP, Noubary F, Freedberg KA, Wilson D, Bishai WR, Rodriguez W, Bassett IV (2014). Evaluating diagnostic point-of-care tests in resource-limited settings. The Lancet Infectious Diseases 14(3):239-249.

Khuroo MS, Khuroo NS, Khuroo MS (2015). Diagnostic accuracy of point-of-care tests for hepatitis C Virus Infection: A systematic review and meta-analysis. Plos One 10(3):e0121450.

Madiou JP (2016). Etude ANRS 12270 : prévalence du VHC/VHB en population générale au Burkina-Faso | Vih.org. Retrieved May 23, 2018, from http://vih.org/20160423/etude-anrs-12270-prevalence-du-vhcvhb-en-population-generale-au-burkina-faso/138077

McHugh ML (2012). Interrater reliability: the kappa statistic. Biochemia Medica 22(3), 276-282. Retrieved from

Montebugnoli L, Borea G, Miniero R, Sprovieri G (1999). A rapid test for the visual detection of anti-hepatitis C virus antibodies in whole blood. Clinica Chimica 288(1-2):91-96.

Moosavy SH, Davoodian P, Nazarnezhad MA, Nejatizaheh A, Eftekhar E, Mahboobi H (2017). Epidemiology, transmission, diagnosis, and outcome of Hepatitis C virus infection. Electronic Physician 9(10):5646-5656.

Nagalo MB, Sanou M, Bisseye C, Kaboré MI, Nebie YK, Kienou K, Kiba A, Dahourou H, Ouattara S, Zongo JD, Simporé J (2011). Seroprevalence of human immunodeficiency virus, hepatitis B and C viruses and syphilis among blood donors in Koudougou (Burkina Faso) in 2009. Blood Transfusion 9(4):419-424.

Njouom R, Tejiokem MC, Zanga MC, Pouillot R, Ayouba A, Pasquier C, Nerrienet E (2006). A cost-effective algorithm for the diagnosis of Hepatitis C virus infection and prediction of HCV viremia in Cameroon. Journal of Virological Methods 133(2):223-226.

O'Connell RJ, Gates RG, Bautista CT, Imbach M, Eggleston JC, Beardsley SG, Manak MM, Gonzales R, Rentas FJ, Macdonald VW, Cardo LJ, Reiber DT, Stramer SL, Michael NL, Peel SA. (2013). Laboratory evaluation of rapid test kits to detect hepatitis C antibody for use in predonation screening in emergency settings. Transfusion 53(3):505-517.

Pruett CR, Vermeulen M, Zacharias P, Ingram C, Tayou Tagny C, Bloch EM (2015). The use of rapid diagnostic tests for transfusion infectious screening in Africa: A Literature Review. Transfusion Medicine Reviews 29(1):35-44.

Scheiblauer H1, El-Nageh M, Nick S, Fields H, Prince A, Diaz S (2006). Evaluation of the performance of 44 assays used in countries with limited resources for the detection of antibodies to hepatitis C virus. Transfusion 46(5):708-718.

Semugoma NP, Rebe K, Sonderup MW, Kamkeumah M, De Swardt G, Struthers H, Eksen H, McIntyre J (2017). Hepatitis C: A South African literature review and results from a burden of disease study among a cohort of drug-using men who have sex with men in Cape Town, South Africa. South African Medical Journal 107(12):1116-1120.

Spearman CW, Dusheiko GM, Hellard M, Sonderup M (2019). Hepatitis C. The Lancet 394(10207):1451-1466.

Tang W, Chen W, Amini A, Boeras D, Falconer J, Kelly H, Peeling R, Varsaneux O, Tucker JD, Easterbrook P (2017). Diagnostic accuracy of tests to detect Hepatitis C antibody: a meta-analysis and review of

the literature. BMC Infectious Diseases 17(Suppl 1):695.

World Health Organization (WHO) (2017). Global Hepatitis Report, *2017.* (p. 82). Geneva. www.who.int/hepatitis/publications/global-hepatitis-report2017/en/

World Health Organization (WHO) (2019). Hepatitis C. Retrieved November 20, 2019, from https://www.who.int/news-room/fact-sheets/detail/hepatitis-c

Zeba MT, Sanou M, Bisseye C, Kiba A, Nagalo BM, Djigma FW, Compaoré TR, Nebié YK, Kienou K, Sagna T, Pietra V, Moret R, Simporé J (2014). Characterisation of hepatitis C virus genotype among blood donors at the regional blood transfusion centre of Ouagadougou, Burkina Faso. Blood Transfusion 12(Suppl 1):s54-s57.

Related Journals:

African Journal of
Biochemistry Research

Journal of
Microbiology and Antimicrobials

African Journal of
Microbiology Research

Journal of
Biophysics and Structural Biology

International Journal of
**Biotechnology and Molecular
Biology Research**

Journal of
Bioinformatics and Sequence Analysis

African Journal of
Biochemistry Research

Journal of
Microbiology and Antimicrobials

African Journal of
Microbiology Research