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MICROBIOLOGICAL QUALITY AND SAFETY ASSESSMENT OF (SOBIA) A TRADITIONALLY FERMENTED BEVERAGE OF WESTERN SAUDI ARABIA

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Abstract– Sobia is a traditional fermented beverage that is well known in the western province of Saudi Arabia particularly during Ramadhan season. The beverage is sold by retailers as well as by anonymous street vendors. We aimed to examine the microbiological qualities of sobia and provide a safety assessment of its consumption. Total counts of aerobic mesophilic bacteria were performed on plate count agar, determination of staphylococci count was carried on mannitol salt agar, while quantitative determination of coliforms and thermotolerant coliforms was carried out by most probable number (MPN) method, antimicrobial susceptibility testing was carried out by means of disc diffusion method on Mueller-Hinton agar. All sobia samples (P <0.001) obtained from anonymous street vendors or low profile retailers were found to be in an unsatisfactory microbiological quality. *Escherichia coli*, *Cronobacter sakazakii*, *Enterobacter cloacae* and *Klebsiella pneumoniae* were predominant than other coliforms genera. Although the majority of these species (P <0.001, x^2) were not exhibiting multidrug resistance, the consumption of sobia samples sold on the street by anonymous vendors may pose significant public health risk and therefore it should be avoided.

INTRODUCTION

Sobia is a traditionally fermented beverage that is popular in western and north western provinces (Makkah and Madinah Provinces) of Saudi Arabia. Traditionally the beverage is popular during the month of Ramadhan and used to be home prepared during the month to be served chilled after iftar (breaking the fast). In the past two decades, there has been a marked increase of the demand for sobia, especially during Ramadhan and thus, it has become an important food commodity during the month. During Ramadhan, sobia is sold in shops by well known authorized retailers and also by anonymous street vendors.

Sobia beverage is prepared by adding water to wheat flour or malt flour or even bread leftover, mixed well and filtered through cheese cloth. The filtrate is transferred to a larger container and sugar is added, additional ingredients also may be added such as cinnamon and cardamom. The ingredients are mixed well and containers are covered well and left at a warm place (30 – 40 °C) for 24 h for the fermentation process (Gassem, 2002). After the fermentation process is complete the beverage is dispensed into plastic containers or plastic bags and ready to be sold. In this respect, risk of microbiological contamination is very likely to occur

during the preparation and/or the packaging process of sobia.

Standard methods for microbiological assessment of the quality and safety of foodstuff require a number of standard analytical tests to be performed (Andrews, 1997; HPA, 2009; FEHD, 2014). Aerobic total plate count (TPC) may provide an estimation of the total number of bacteria in a given sample, thus it can indicate the overall microbiological quality of a given product and also used as an indicator of the sanitary conditions under which foodstuff was produced, processed and/or stored (Andrews, 1997; HPA, 2009; FEHD, 2014). The coliform group has been widely used to assess the microbiological quality of drinking water and foodstuff for many years, the group comprises of 32 genera, such as *Klebsiella*, *Enterobacter*, *Citrobacter*, *Serratia* and *Escherichia* (Leclerc *et al.*, 2001), although not all members of the coliform group are exclusively of fecal origin, it is believed that the coliform group is used as an indicator for the presence of enteric pathogens in water and food (Leclerc *et al.*, 2001). Thus the thermotolerant coliform group, which comprises mainly of *Escherichia coli* is exclusively of fecal origin, therefore is a more reliable indicator for the presence of pathogens in foodstuff (Andrews, 1997; HPA, 2009; FEHD, 2014). The presence of *Staphylococcus aureus* in food indicate contamination of foodstuff from the skin, nose and mouth of food handlers and suggest inadequate sanitary conditions (Andrews, 1997; HPA, 2009; FEHD, 2014).

Microbiological properties of sobia beverage are still not yet fully explored, Gassem (2002) examined the overall microbial composition of sobia beverage and concluded that sobia is a lactic acid fermentation product due to the predominance and diverse lactic acid bacterial genera found in the samples that were examined. In addition to lactic acid bacteria, various coliforms (e.g. *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae* and *Cronobacter sakazakii*), yeast and mold species were also present in sobia beverage (Gassem, 2002). More recently, El Said (2019) examined ten samples for their hygiene qualities and reported the presence of *E. coli*, *Cronobacter sakazakii*, *Enterobacter cloacae*, *Klebsiella pneumoniae* and other genera with low resistance to antimicrobial agents. It was concluded that the possibilities of acquiring enteric infections from sobia beverage cannot be ruled out (El Said, 2019).

Thus in the current study we aimed to explore and assess the safety and microbiological quality of sobia beverage sold in authorized retailers as well as street vendors within the cities of Makkah and Jeddah, western Saudi Arabia.

MATERIALS AND METHODS

Sampling

A total number of 20 sobia samples were purchased from various locations within Makkah and Jeddah cities during the month of Ramadhan 2021 (April-May, 2021). A brief description of each sample is given in Table 1. Samples were transported to the laboratory on ice, away from sunlight and microbiological examinations were performed as the same day of sampling.

Bacteriological analysis

The bacteriological qualities of each sobia sample were assessed by means of; total plate count of aerobic mesophilic bacteria on plate count agar (HiMedia, Mumbai, India), samples were serially diluted, and a volume of 1.0 ml of dilution 10^{-2} of each sample was inoculated into petri dish (each samples was examined in duplicates) using the pour plate method, incubation was carried out at 35 °C for 24-48 hrs (Gassem, 2002; Adebayo-Oyetoro *et al.*, 2013).

Quantitative determination of coliforms in each sobia sample was performed by means of the most probable number (MPN) method of five replicates of 10 ml, 1.0 ml and 0.1 ml of each sample using MacConkey broth (Oxoid, Basingstoke, UK), incubation was at 37 °C for 24-48 hrs (Bartram and Pedley, 1996; Salman and Hamad, 2011). As for the determination of fecal and/or thermotolerant coliforms, the same procedure of coliforms was carried out, however the incubation was achieved at 44.5 °C for 24-48 hrs (Bartram and Pedley, 1996; Andrews, 1997).

Determination of the total count of staphylococci was carried out by pour plate technique using mannitol salt agar (HiMedia), using 1.0 ml of 10^{-2} dilutions. Plates were incubated at 37 °C for 24-48 hrs (Andrews, 1997; Organji *et al.*, 2018).

Identification and confirmation of presumptive isolates

Identification of coliform genera and *E. coli* was achieved by streaking a loopful of positive MacConkey broth tubes of the MPN test on

MacConkey (Mast Diagnostics, Bootle, UK) and eosin methylene blue agar (EMB) (HiMedia) plates. Plates were incubated at 37 °C for 24 hrs. Identification of coliform genera was performed by API 20E strips (BioMerieux, Marcy-I'Etoil, France) (Gassem, 2002) and Viteck® 2 compact system (BioMerieux) (El Said, 2019) according to the manufacturer's instructions. Phenotypic detection of hemolysins of each isolate was carried out by streaking of each identified isolate on blood agar base (Mast Diagnostics) supplemented with 5 % sheep blood (Oxoid). Plates incubated at 37 °C for 24-48 hrs.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing using disc diffusion technique, i.e. Kirby-Bauer method (Abulreesh *et al.*, 2017; EUCAST, 2021) was carried out to examine the resistance patterns of each of the identified coliform genera against 12 antibiotics (Oxoid) belonging to eight antimicrobial agent classes as follows: ticarcillin 75 µg, piperacillin 30 µg (penicillins), cefepime 30 µg, ceftazidime 10µg (cephalosporins), imipenem 10µg, meropenem 10 µg (carbapenems), aztreonam 30 µg (monobactams), ciprofloxacin 5 µg (fluoroquinolones), amikacin 30 µg, gentamicin 10 µg (aminoglycosides), tigecycline 15 µg (tetracyclines) and chlorampenicol 30 µg (amphenicols). Antimicrobial susceptibility testing was carried out by plating a lawn of a 0.5 McFarland suspension of each isolates on Mueller-Hinton agar (HiMedia) plates and incubation was achieved at 37 °C for 18 hrs (Abulreesh *et al.*, 2017; EUCAST, 2021). Interpretation of the antimicrobial susceptibility breakpoints was based on the guidelines of the European Committee on Antimicrobial Susceptibility testing (EUCAST, 2021). .

RESULTS

A total of twenty sobia samples obtained from various anonymous street vendors and retailers were examined for their microbiological quality. As shown in Table 2, the majority of the samples (80%, n = 20) were of unsatisfactory (coliforms exceeds 10^4) (Fig. 1) and/or moderate (colifomrs more than 100 MPN 100 ml^{-1}) microbiological qualities, thus significant number (P <0.001, Chi-squared) were of unacceptable microbiological qualities. Samples SOB1 and SOB16 were of acceptable quality (coliforms <2 MPN 100 ml^{-1}) (Fig. 2), these samples has proper packaging with proper product label that claim the product is factory manufactured, not like other samples prepared at homes.

Table 1. Brief description of sobia samples analyzed in this study

Sample code	Place of purchase	Packaging	pH	City
Sob1†	Retailer	Plastic bottle	3.56	Makkah
Sob2	Anon. street vendor	Plastic bag	3.73	Makkah
Sob3	Anon. street vendor	Plastic bag	3.37	Makkah
Sob4	Anon. street vendor	Plastic bag	3.81	Makkah
Sob5	Anon. street vendor	Plastic bag	3.30	Makkah
Sob6	Anon. street vendor	Plastic bag	3.88	Jeddah
Sob7	Anon. street vendor	Plastic bag	4.04	Jeddah
Sob8	Anon. street vendor	Plastic bag	3.66	Makkah
Sob9	Anon. street vendor	Plastic bag	3.90	Makkah
Sob10	Anon. street vendor	Plastic bag	3.85	Jeddah
Sob11	Anon. street vendor	Plastic bag	4.15	Makkah
Sob12	Retailer	Plastic bottle	4.04	Makkah
Sob13	Anon. street vendor	Plastic bag	3.92	Makkah
Sob14	Anon. street vendor	Plastic bag	3.74	Makkah
Sob15	Anon. street vendor	Plastic bag	4.14	Makkah
Sob16†	Retailer	Plastic bottle	2.99	Makkah
Sob17	Retailer	Plastic bottle	4.08	Jeddah
Sob18	Retailer	Plastic bottle	4.12	Jeddah
Sob19	Retailer	Plastic bottle	3.77	Makkah
Sob20	Retailer	Plastic bottle	3.79	Makkah

Anon. = Anonymous

† = These two samples obtained from a well-known sobia retailer that has been practicing this for the past 30 years. The bottles has proper product label and it is a factory product, not home made.

With regards to thermotolerant coliforms, 85 % of the twenty sobia samples (P <0.001, Chi-squared) were exceeding the acceptable limits for thermotolerant coliforms (*E. coli*) (less than 20 CFU ml^{-1}). *Escherichia coli* were detected in five of the samples yielding higher levels of thermotolerant coliforms (>2400 MPN 100 ml^{-1}), particularly in samples SOB7, SOB11, SOB15, SOB17 and SOB18 (Table 2).

Determination of staphylococci in general and *Staphylococcus aureus* in particular total counts in sobia were negative in all samples. No growth was

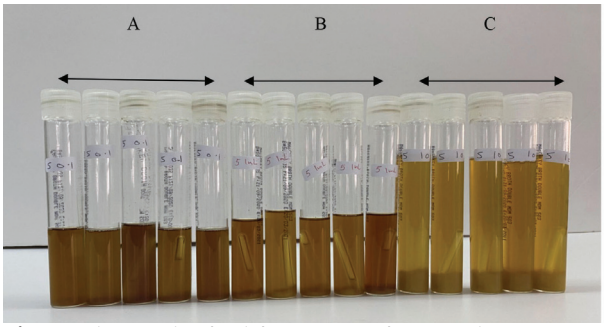

Fig. 1. The result of coliform MPN, for sample SOB17, as seen all (**A**) five tubes inoculated with 0.1 ml, all (**B**) five tubes inoculated with 1.0 and all (**C**) five tubes inoculated with 10 ml of the samples were positive for acid and gas, which, according to MPN tables indicate that number of coliform bacteria is >2400 100 ml^{-1}. Similar results were observed with samples SOB3, SOB4, SOB6, SOB7, SOB8, SOB10, SOB11, SOB14, SOB15 and SOB18.

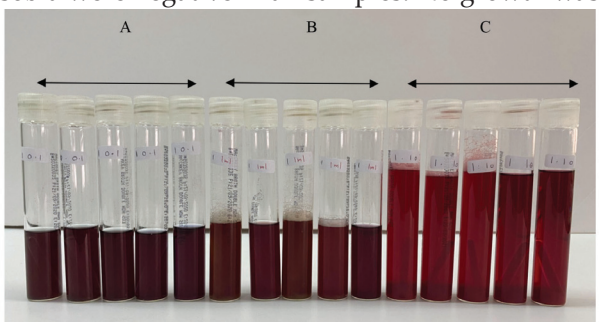

Fig. 2. The result of coliform MPN, for sample SOB16, as seen all (**A**) five tubes inoculated with 0.1 ml, all (**B**) five tubes inoculated with 1.0 and all (**C**) five tubes inoculated with 10 ml of the samples were negative for acid and gas, which, according to MPN tables indicate that number of coliform bacteria is <2 100 ml^{-1}. Similar result was observed with sample SOB1.

Table 2. Bacteriological assessment of sobia samples

Sample	Aerobic total count (CFU ml^{-1})	Coliforms (MPN index 100 ml^{-1})	Thermotolerant coliforms (MPN index 100 ml^{-1})	Detection of *E. coli*	Staphylococcal plate count (CFU ml^{-1})
SOB1	1.22 x 10^5	<2	<2	-	ND
SOB2	5.0 x 10^5	170	1600	-	ND
SOB3	4.5 x 10^5	≥2400	1600	-	ND
SOB4	3.0 x 10^5	≥2400	≥2400	-	ND
SOB5	5.0 x 10^5	≥350	350	-	ND
SOB6	8.5 X 10^5	≥2400	1600	-	ND
SOB7	7.1 X 10^5	≥2400	≥2400	+	ND
SOB8	6.1 x 10^5	≥2400	79	-	ND
SOB9	5.1 x 10^5	≥540	540	-	ND
SOB10	4.5 x 10^5	≥2400	170	-	ND
SOB11	6.8 x 10^5	≥2400	≥2400	+	ND
SOB12	8.1 x 10^5	≥540	540	-	ND
SOB13	6.6 x 10^5	≥540	540	-	ND
SOB14	9.0 x 10^5	≥2400	≥350	-	ND
SOB15	1.7 x 10^6	≥2400	≥2400	+	ND
SOB16	1.3 x 10^5	<2	<2	-	ND
SOB17	1.5 x 10^6	≥2400	≥2400	+	ND
SOB18	1.2 x 10^6	≥2400	≥2400	+	ND
SOB19	5.9 x 10^5	34	5	-	ND
SOB20	7.2 x 10^5	23	<2	-	ND
P†		<0.001			
P‡			<0.001		

P is from Chi-squared test to test the hypothesis that all samples are of satisfactory microbiological qualities according to the coliform (P†) and thermotoletant coliform (P‡) MPN index. ND = not determined

observed on mannitol salt agar inoculated with 1.0 ml of serially diluted samples (Table 2).

A total of 29 isolates belonging to the *Enterobacteriaceae* were identified from sobia samples (with exceptions of samples SOB1 and SOB16), The twenty nine isolates were belonging to six taxa; *Klebsiella pneumoniae* (8), *Enterobacter cloacae* (6), *Escherichia coli* (5), *Cronobacter sakazakii* (5), *Klebsiella oxytoca* (2), *Enterobacter aerogenes* (1), *Citrobacter freundii* (1) and *Serratia plymuthica* (1) (Table 3).

Antimicrobial susceptibility profiles of the coliform genera are shown in Table 3. The highest resistance was shown against ceftazidime (93.11 %, n = 29) while resistance was low (31.1 %) to cefepime which is another agent belong to cephalosporins. However resistance to ticarcillin was exhibited by 21 (74.2 %, n = 29) of the confirmed isolates, followed by resistance to piperacillin (58.6 %), which are both penicillins. Conversely, no resistance was observed among all coliform genera against imipenem and meropenem (carbapenems), amikacin and gentamicin (aminoglycosides) tigecycline (tertacyclines) and chloramphenicol (amphenicols) (Table 3).

Multidrug resistance (resistance to three or more antimicrobial agents) was observed in 20.7 % of the 29 genera, which is significantly low (P <0.001, Chi-squared) as listed in Table 4. Multidrug resistant genera were *Klebsiella pneumoniae* (2), *Enterobacter cloacae* (2), *Klebsiella oxytoca* (1), and *Citrobacter freundii* (1). All *Escherichia coli* and *Cronobacter sakazakii* isolates were resistant to penicillins and/or cephalosporins. Moreover, none of the twenty nine coliform genera were possessing hemolysisns as no hemolytic activities were observed on blood agar plates (Table 4).

DISCUSSION

Generally, fermented food and beverages have been opted for in order to preserve perishable commodities and extend their shelf life. Over the years, fermented beverages have become part of the cultural identity of the communities and areas of their origins, some of which have further economic advantages. Thus, over the years, sobia has become an important part of the culture and the economics of residents of the western province of Saudi Arabia particularly during Ramadhan season, which later introduced to other provinces within the country. Also the beverage is well known and part of the culture of the people of Egypt.

Table 3. Antimicrobial susceptibility profiles of coliform genera isolated from sobia

Coliform genera	Total number of isolate	Resistance profiles											
		TC	PRL	IMI	MEM	CIP	CPM	CAZ	TIG	ATM	GM	AK	C
E. coli	5	4	3	0	0	0	3	5	0	0	0	0	0
Enterobacter cloacae	6	3	2	0	0	0	1	6	0	2	0	0	0
Enterobacter aerogenes	1	1	1	0	0	0	0	1	0	0	0	0	0
Cronobacter sakasakii	5	4	2	0	0	0	3	3	0	0	0	1	0
Klebsiella pneumoniae	8	7	7	0	0	0	1	8	0	2	0	0	0
Klebsiella oxytoca	2	2	2	0	0	0	1	2	0	0	0	0	0
Citrobacter freundii	1	0	0	0	0	1	0	1	0	1	0	0	0
Serratia plymuthica	1	0	0	0	0	0	0	1	0	0	0	0	0
Total	29	21	17	0	0	1	9	27	0	5	0	1	0

TC = ticarcillin, PRL = piperacillin, IMI = imipenem, MEM = meropenem, CIP = ciprofloxacin, CPM = cefepime, CAZ = ceftazidime, TIG = tigecycline, ATM = aztreonam, GM = gentamicin, AK = amikacin, C = chloramphenicol

Given that sobia is a fermented beverage, its microbiological quality cannot be measured by aerobic total plate count, however, we performed the test to get an idea of the total aerobic, mesophilic bacteria that may play a role in the fermentation process. Gassem (2002) reported 4.17 to 8.1 log CFU ml^{-1} of total plate count of aerobic mesophilic bacteria in sobia samples, which is similar to our results. Although we did not perform total counts of lactic acid bacteria, the results reported in the study of Gassem (2002) suggests that lactic acid bacteria in sobia constitute significant number among the aerobic mesophlic genera. It is generally accepted that traditionally fermented beverages may contain higher total bacterial counts that usually originate from the material or the water used to prepare these beverages and may play, in part, a role in the fermentation process (Ilango and Antony, 2014; Anal, 2019).

The incidence of coagulase-negative staphylococci in fermented foodstuff usually may not suggests health hazards given that some species are usually involved in fermentation process (Organji *et al.*, 2018), while the presence of *Staphylococcus aureus* in foodstuffs may indicate lack of hygiene practices and cross contamination from food handlers and may constitute health risks due to staphylococcal food poisoning (Abulreesh and Organji, 2011), however, in this study, all sobia samples were negative for the presence of staphylococci in general and *Staphylococcus aureus* in particular, this is perhaps due to the very low pH of most of the samples that was recorded below pH 4.0. It is well established that the favorable pH value of staphylococci ranged between pH 4 to 8, thus the high acidity (pH below 4) observed in sobia samples may have hindered the growth of all species belonging to staphylococci, furthermore, given that the Sobia beverage is in fact a lactic acid fermentation beverage as suggested by previous study (Gassem, 2002), and among the lactic acid bacterial genera that can be found in sobia are *Lactobacillus plantarum*, *Lactobacillus brevis* and other genera of *Lactobacillus* (Gassem, 2002), these species may produce bacteriocin which, may, in part, contribute to the absence of staphylococci even in samples that have pH 4 or above (Kivanc and Yapici, 2019).

The detection of coliforms in foodstuffs generally suggests low hygienic qualities. However, according to standards and guidelines of safe foods, edible commodity are classified into three categories based on the levels of detectable (culturable) coliforms; (i)- satisfactory, when coliforms presents in numbers less than 10^2 CFU gm^{-1}, (ii)- borderline, when coliform counts ranged between 10^2 to 10^4 gm^{-1} and (iii) unsatisfactory when 1.0 gram of food yield more than 10^4 CFU of culturable coliforms (Anrews, 1997; HPA, 2009; FEHD, 2014). In the current study, with the exception of samples SOB1 and SOB16, significant numbers of sobia samples (P <0.001, x^2) were found to yield coliforms above 2400 MPN 100 ml^{-1} and that may suggest that these samples have unsatisfactory microbiological quality and may indicate low quality of materials used for the preparation of sobia and/or lack of hygiene practices during the preparation steps.

Similarly, the numbers of thermotolerant coliforms (if confirmed as *E. coli*) can also give an indication of low food hygienic qualities and may suggests recent fecal contamination from humans or warm blooded animals (Abulreesh *et al.*, 2004), thermotolerant coliforms and *E. coli* in particular should not exceed 20 CFU g^{-1} in food classified under the satisfactory quality, while more 10^2 CFU g^{-1} of *E. coli* render foodstuff unsatisfactory and not suitable for consumption due to the possibilities of acquiring enteric infections (Andrews, 1997; HPA, 2009; FEHD, 2014). Significantly large number of samples (P <0.001, x^2) yielding thremotolerant coliforms levels of more than 10^2 even more than 10^4 MPN 100 ml^{-1} and *E. coli* was detected in five of the samples suggesting that these samples are fecally contaminated and its consumption constitute health risks of acquiring diarrhea diseases (Kassem *et al.*, 2020).

In the current study, various genera belonging to the colifrom group were identified from sobia samples; *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Escherichia coli*, and *Cronobacter sakazakii* were frequently detected, these results are in agreement with Gassem (2002) and El Said (2019) who reported the predominance of these species in sobia samples. Antimicrobial susceptibility patterns of these isolates suggest low prevalence of multidrug resistant strains (P <0.001, x^2), which is also in agreement with the results of El Said (2019), however their presence in sobia may have serious public health implications.

Klebsiella pneumoniae were more prevalent in sobia samples examined in this study, although the majority of these isolates were not multidrug resistant and did not produce hemolysis on blood

agar plates, its presence in sobia and other food commodities may pose public health risks. *Klebsiella pneumoniae* is an opportunistic pathogen that is ubiquitous in nature and causes bacteriema, respiratory and urinary tract infections (Effah *et al*., 2020; Jasim and Farhan, 2020), although *K. pneumoniae* is not considered as foodborne pathogen (Hartantyo *et al*., 2020), however, its presence in food and beverages, particularly that is sold by street vendors and other retailers has received considerable attention worldwide recently. It was suggested that their presence in food should not be underestimated, particularly as source of antibiotic resistant and virulence factors genes for other foodborne pathogens or food-associated microflora (Mousse *et al*., 2016; Zhang *et al*., 2018; Hartantyo *et al*., 2020).

Enterobacter cloacae is found in various ecological niches, such as water, soil as well as the intestinal tract (Leclerc *et al*., 2001), it was the second most prevalent species to be identified in sobia samples in the current study. *Enterobacter cloacae* has also been considered as an opportunistic pathogen that has been associated with different nosocomial infections (Davin-Regli and Pages, 2015). *Enterobacter cloacae* has been frequently detected in food samples from street vendors (Haryani *et al*., 2008; Manhique *et al*., 2020), road-side food halls and outlets (Nyenje *et al*., 2012) and even sobia samples (Gassem, 2002; El Said, 2019). Despite the fact that *E. cloacae* reported in this study not exhibiting multiple drug resistant patterns, their presence in sobia samples suggests low hygiene conditions of the beverage during the preparation and/or packaging (Nyenje *et al*., 2012; Manhique *et al*., 2020), therefore, the consumption of such beverage may pose public health threats.

Escherichia coli is a common inhabitant of the intestinal tract of humans and warm-blood animals

Table 4. Multidrug-resistance patterns and hemolysins production of coliform genera isolated from sobia

Coliform genera	Origin	Resistance patterns	Number of classes	Type of blood hemolysis
E. coli	SOB7	PRL, TC, CAZ, CPM	2	(no hemolysis)
E. coli	SOB11	CAZ	1	(no hemolysis)
E. coli	SOB17	PRL, TC, CAZ	2	(no hemolysis)
E. coli	SOB18	PRL, TC, CAZ, CPM	2	(no hemolysis)
E. coli	SOB18	TC, CAZ, CPM	2	(no hemolysis)
Klebsiella pneumoniae	SOB2	PRL, TC, CAZ, ATM	3	(no hemolysis)
Klebsiella pneumoniae	SOB5	PRL, CAZ, ATM	3	(no hemolysis)
Klebsiella pneumoniae	SOB10	PRL, TC, CAZ	2	(no hemolysis)
Klebsiella pneumoniae	SOB11	PRL, TC, CAZ	2	(no hemolysis)
Klebsiella pneumoniae	SOB11	PRL, TC, CAZ, CPM	2	(no hemolysis)
Klebsiella pneumoniae	SOB15	TC, CAZ, CPM	2	(no hemolysis)
Klebsiella pneumoniae	SOB17	PRL, TC, CAZ	2	(no hemolysis)
Klebsiella pneumoniae	SOB18	PRL, TC, CAZ	2	(no hemolysis)
Enterobacter cloacae	SOB2	TC, ATM, CAZ	3	(no hemolysis)
Enterobacter cloacae	SOB5	TC, PRL, ATM, CAZ	3	(no hemolysis)
Enterobacter cloacae	SOB12	CAZ	1	(no hemolysis)
Enterobacter cloacae	SOB15	TC, PRL, CAZ	2	(no hemolysis)
Enterobacter cloacae	SOB17	CAZ	1	(no hemolysis)
Enterobacter cloacae	SOB18	PRL, CAZ	2	(no hemolysis)
Cronobacter sakasakii	SOB4	PRL, CPM	2	(no hemolysis)
Cronobacter sakasakii	SOB7	TC, CPM	2	(no hemolysis)
Cronobacter sakasakii	SOB9	TC, PRL	1	(no hemolysis)
Cronobacter sakasakii	SOB12	TC, PRL	1	(no hemolysis)
Cronobacter sakasakii	SOB17	AK, CPM	1	(no hemolysis)
Klebsiella oxytoca	SOB3	PRL, TC, CAZ, ATM	3	(no hemolysis)
Klebsiella oxytoca	SOB13	PRL, TC, CAZ, CPM	2	(no hemolysis)
Enterobacter aerogenes	SOB18	PRL, TC, CAZ	2	(no hemolysis)
Citrobacter freundii	SOB3	ATM, CAZ, CIP	3	(no hemolysis)
Serratia plymuthica	SOB17	CAZ	1	(no hemolysis)
P			<0.001	

P is from Chi-squared to test the hypothesis that multidrug-resistant coliform genera in sobia are more than non-multidrug-resistant genera

TC = ticarcillin, PRL = piperacillin, CIP = ciprofloxacin, CPM = cefepime, CAZ = ceftazidime, ATM = aztreonam, AK = amikacin,

(Leclerc *et al.*, 2001), it is exclusively of fecal origin, thus its presence in food, drinking water, recreational water and soil suggests recent fecal contamination (Abulreesh *et al.*, 2004; Odonkor and Ampofo, 2013, Kassem *et al.*, 2020). Five of the sobia samples examined in the current study harbored *E. coli*, those samples were from anonymous street vendors which is in accordance with the results reported previously by Gassem (2002) and El Said (2019), although none of the *E. coli* isolates were exhibiting multidrug resistance patterns and showed no hemolytic activity, which is a virulence factor for pathogenic strains, the sobia samples with *E. coli* constitute a major public health risk to the consumers given the possibility of acquiring foodborne infections due to fecal contamination.

Cronobacter sakazakii (formerly known as *Enterobacter sakazakii*) is another member of the *Enterobacteriaceae*, although the exact natural habitat of *C. sakazakii* is unknown, it is believed to be widely distributed in diverse environmental niches, such as water, soils and frequently found on the surfaces of plants and vegetables (Zhou *et al.*, 2012). *Cronobacter sakazakii* is an established foodborne pathogen, particularly associated with infants dairy formulas fatal infections, the bacterium has been associated with enterocolitis, sepsis and meningitis in preterm neonatal infants (Fakurddin, 2013, Parra-Flores *et al.*, 2018). *Cronobacter sakazakii* is among the emerging foodborne pathogens and it has been considered by food safety authorities worldwide in their guidelines and standard methods for microbiological safe food (HPA, 2009), this perhaps due to their low infective dose that has been proposed around 10^3 to 10^4 CFU g-1 (Fakruddin, 2013). In the current study *C. sakazakii* was detected in some of the sobia samples, which also was reported by El Said (2019) in previous his study on sobia. Although none of the isolates were susceptible to most of the antimicrobial agents, their presence in sobia samples suggests potential health hazards for the consumption of this beverage.

CONCLUSION

With the exception of two samples that were originate from well known sobia retailer with proper packaging and proper product label, that probably produced with good manufacturing code of practice, sobia beverage that are sold by anonymous street vendors and some low profile retailers have shown to be in an unsatisfactory microbiological quality, as well as harboring various coliform genera that are indicative of recent fecal contamination (e.g. *E. coli*) and/or foodborne pathogens (e.g. *C. sakazakii*), thus the consumption of sobia beverage that are sold anonymously in the street should be avoided due to possible associated public health risks.

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