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Full Length Research Paper

Effectiveness of commonly used antiseptics on bacteria causing nosocomial infections in tertiary hospital in Malaysia

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The antimicrobial inhibitory effects of five common antiseptics [Chlorhexidine (CHX), Hydrogen peroxide (H_2O_2), Iodine, Ethanol and Dettol] were investigated using agar well diffusion method. The organisms used included methicillin-resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumannii, Escherichia coli*, *Klebsiella* species and *Pseudomonas aeruginosa*. The undiluted concentrations of the antiseptics showed variable zones of inhibition against the tested organisms, on MRSA it ranged from 25 mm (CHX) to 30 mm in other antiseptics, on *A. baumannii* 20 mm (CHX) to 34 mm Dettol, on *E. coli* 20 mm Dettol to 38 mm (H_2O_2), on *Klebsiella* spp. 20 mm Dettol to 24 mm (CHX), whereas on *P. aeruginosa* it ranged from 13 mm Iodine to 30 mm (H_2O_2). The minimal inhibitory concentration (MIC) of chlorhexidine concentration against MRSA and *P. aeruginosa* was 10%, while *A. baumannii* was 20%. All the study bacteria were resistant to ethanol by all concentrations. The result showed that H_2O_2 was the most effective antiseptics than the others followed by CHX. The study bacteria were found to be crucially susceptible to the routinely used antiseptics tested. Though, there is the need for continuous surveillance for the detection of emerging resistance pattern.

Key words: Antimicrobial, antiseptics, disinfectants, nosocomial.

INTRODUCTION

Nosocomial infections (NI) are referred to those infections occurring after 48 h of hospital admission, or 3 days of discharge (Kouchak and Askarian, 2012). About 10% of the hospital admitted persons will have NI, and it has been shown that NI is usually associated with prolong length of hospital stay, increased costs, and resulted in

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significant morbidity and mortality (Al-Talib et al., 2010; Raines and Rosen, 2016). Currently, NI has become a trend in healthcare setting globally including Malaysia. Nosocomial bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*, have become endemic in many health care centres. Infections with these organisms are often difficult to treat, owing to a reducing armamentarium of active antiseptic agents. Also, hospital associated infections involving these and other microorganisms are associated with considerable morbidity and mortality (Climo et al., 2013). Antiseptics and disinfectants had a fundamental role in infection control practices and help in the avoidance of NI. Antiseptics are used in sterilization of medical and surgical instruments and wards equipment. However, extensive using of different antiseptics might lead to the development of resistant pathogens that eventually makes the antiseptics become ineffective (Matthew et al., 2017). Different bacteria showed variable degrees of resistance to antiseptics (McDonnell and Russell, 1999); although, Gram-negative bacteria are commonly more resistant than Gram-positive bacteria to antiseptics (Russell, 1999). Antiseptics are mainly used to inhibit the growth of microorganisms or to interrupt the route of transmission of germs between the infection source and healthy subjects (Mbajiuka et al., 2015). Previous study by El-Mahmood and Doughari (2009) revealed that five frequently used antiseptics were contaminated with nosocomial Gram positive and negative bacteria, therefore, antiseptics used in hospitals and laboratories must be evaluated regularly to determine their potency validation to remove or inactivate known pathogens from inanimate objects (Sridhar, 2012). Chlorhexidine is an antiseptic with a broad-spectrum activity against many organisms, including *S. aureus* and *Enterococcus* species. Chlorhexidine is a cationic polybiguanide that has antibacterial effects and has been used as antiseptic in clinical practice (Mullany et al., 2006). Chlorhexidine salts dissociate and release the positively charged chlorhexidine cationic molecules which bind to negatively charged bacterial cell walls and causing bactericidal effect (Cheung et al., 2012). Using chlorhexidine at low concentrations resulted in a bacteriostatic effect while at high concentrations it can cause membrane disruption and cell death. Chlorhexidine lasts much longer than other antiseptics, therefore, it is often combine with alcohol in skin preparation to reduce microbial burden on patients' skin and prevent secondary bacterial infections (Climo et al., 2013). Previous studies have found that daily bathing with 2% chlorhexidine-impregnated washcloths reduced the incidence of NI infections by 60% (Climo et al., 2013; Vernon et al., 2006).

Hydrogen peroxide (H_2O_2) plays a central role in sterilization and disinfection of critical items in Malaysian hospitals. Also H_2O_2 is the most effective antiseptic used in hospitals since the 1920s because it kills bacteria cells by destroying their cell walls. H_2O_2 has "hydroxyl radicals" a potent oxidant, which react with macromolecules such as membrane lipids and DNA thus resulting in bacterial death (Shahriari et al., 2011). In its pure form, H_2O_2 is a colourless liquid, slightly more viscous than water. H_2O_2 is used in hospital and ICU in a vapour form to decontaminate rooms from multi-drug resistant, also used to sterile surfaces, including surgical tools (Lemmen et al., 2015).

Nowadays most of the hospital used Iodine (povidone iodine) which is a natural dark violet, non-metallic solution that considered among the most effective skin antiseptics and used widely in minor wound cleaner. Iodine has excellent bactericidal, fungicidal, tuberculocidal, virucidal and sporicidal properties (Bouaziz et al., 2016). Iodine can penetrate the cell wall of microorganisms quickly, and the lethal effects are believed to result from disruption of protein and nucleic acid structure and synthesis (McKeen, 2012). Although, povidone-iodine has a rapid bactericidal effect than chlorhexidine, but povidone-iodine has not been shown to have a persistent effect like chlorhexidine (Bigliardi et al., 2017).

Ethanol is used extensively in the homes, healthcare settings and laboratories. It consists of two water-soluble chemical compounds ethyl alcohol and isopropyl alcohol that have germicidal characteristics. Alcohols showed bactericidal rather than bacteriostatic activities against vegetative forms of bacteria but do not destroy bacterial spores. Hence, alcohol is not generally being used as sterilizing material instrument (Tuhina et al., 2013). Both ethanol and isopropanol have similar modes of action against different types of microorganisms, however isopropyl alcohol is likely to be more effective than ethanol against bacteria, while the reverse appears to be true for viruses (William et al., 2008). Dettol is another antiseptic, which is used in hospitals and homes; it is available in multi-forms like soap, spray, hand wash, surface wipes, mildew remover and a bathroom cleaner. The active ingredient in Dettol is para-chloro-meta-xylenol. Dettol has greater effects against Gram-positive bacteria and works by disruption of the cell wall and inhibiting the function of enzymes (Mahon et al., 2014).

The aim of this study was to evaluate the antimicrobial effects of some commonly used disinfectants and antiseptics against common bacteria that cause nosocomial infections in hospitals.

MATERIALS AND METHODS

Antiseptics

This study was conducted in microbiology laboratory at Institute of Medical Molecular Biotechnology, Faculty of Medicine, Universiti Teknologi MARA (UiTM) from February to August 2017. In this study, the same antiseptics which were already used by different wards and Operation Theater in UiTM Private Specialist Centre (PPP-UiTM), Sungai Buloh, Selangor, Malaysia were used. Five commonly used antiseptics and disinfectants were evaluated in this study including Heptin [Chlorhexidine Gluconate 0.5% in alcohol 70% Nanz Med Science Pharma, Himachal Pradesh, India)],

Hydrogen peroxide 6% w/v (Wellmex Sdn Bhd, Selangor, Malaysia), Iodine [Povidone Iodine 7.5% w/v (Thermalife, Pinang, Malaysia)], Alcohol [Ethanol 70% v/v Fisher, Loughborough, UK)] and Dettol [Chloroxylenol 4.8% w/v (Reckitt Benckiser, Hull, UK)].

Disinfectant dilution methods

A series of decreasing concentrations of the antiseptics were obtained using serial dilution method in which the original concentration of antiseptic was considered 100%, the subsequent concentration was prepared by adding 9 ml of antiseptic into a tube with 1 ml distilled water to give 90% concentration. Then the rest concentrations were prepared in descending same manner. The antiseptics concentrations used in this study range from 100 to 10%.

Cultivation of bacterial strains

Five bacterial types isolated from UiTM Private Specialist Centre were used in this study including methicillin-resistant *S. aureus* (MRSA), *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella* species and *P. aeruginosa*. Each bacterium was cultured in nutrient agar for 24 h at 37°C. All tested bacteria were maintained in nutrient broth at 4°C and subcultured on Luria Bertani agar plates 24 h prior to any antimicrobial test. Luria Bertani broth was used for all antibacterial testing.

Antimicrobial susceptibility assays

Well diffusion method

Agar well diffusion method was used to determine antimicrobial activity of different antiseptics. Two bacterial colonies were inoculated in Tryptic soy broth for 3 h at 37°C and turbidity was adjusted in phosphate buffered saline to 0.5 McFarland's scale. 100 µl of bacterial broth was spread on Muller-Hinton agar plates containing ten 6 mm wells. Thirty microliters of each different concentration (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100%) of each antiseptic was poured into each well and these plates were incubated at 37°C aerobically for 24 h. The diameter of the zone of growth inhibition around the wells were measured in millimeters and recorded. Wells containing antiseptics which showed no inhibition zones were considered as negative results. Antibiotic disc was used as positive control.

Minimal inhibitory concentration (MIC)

Broth dilution assay was used to determine the MIC of different antiseptics against bacteria causing nosocomial infections as recommended by the Clinical Laboratory Standards Institute (Wayne, 2012). The concentrations of the antiseptics tested ranged from 100 to 10%. This test was performed in sterile bijou bottles which were loaded with 100 µL of each antiseptic dilution into each bottle.

Bacterial inoculums (100 µL) containing 5×10^5 CFU of each microorganism were added to each bottle (European Society of Clinical Microbiology and Infectious Diseases, 2003). In each panel of the tested antiseptic, a positive control (without antiseptic) and negative control (no inoculum) were added. All bottles were aerobically incubated at 37°C. After incubation for 24 h, the bacterial growth was assayed by its visible turbidity. The highest dilution of the antiseptic which showed no visible bacterial growth and no turbidity in bijou bottle was considered as MIC. After 24 h of incubation, 100 µL of each mixture was pipetted and inoculated on blood agar and spread uniformly with the sterile spreader and again incubated for 24 h at 37°C. On the next day, all blood agars were examined and all bacterial colonies were counted and recorded.

Ethical approval

The study was conducted in accordance with the principles of the Declaration of Helsinki. All participants provided written informed consent.

RESULTS

The antibacterial effect of antiseptics on nosocomial bacteria was presented in Table 1 which showed the inhibitory effects of different antiseptics on nosocomial bacteria using different concentrations of antiseptics. Specific antibiotic discs were used as a positive control accordingly. H_2O_2 showed excellent inhibitory effects on all nosocomial bacteria even with lower concentrations (10%). However, ethanol did not show any inhibitory effects as shown in Figure 1a to c.

The MIC of chlorhexidine against MRSA and *P. aeruginosa* was 10%, while for *A. baumannii* was 20% (Table 2). The results showed that *Klebsiella* spp. was sensitive to H_2O_2 only with MIC of 10%, while *A. baumannii* was resistant to ethanol only. MRSA, *A. baumannii* and *E. coli* were sensitive to Iodine at various concentrations. *E. coli* was sensitive to H_2O_2 with MIC of 50%. Vancomycin disc was used as a positive control and showed inhibition in the growth of MRSA while polymyxin B disc was effective against *A. baumannii* and *P. aeruginosa.* Also, Imipenem showed inhibition of *E. coli* and *Klebsiella* spp.

Thus, H_2O_2 clearly shows effectiveness against all nosocomial bacteria since it has the largest zone of inhibition among all the antiseptics (Table 1).

Table 3 shows the inhibitory effects of the highest concentration of different antiseptics used on nosocomial bacteria after 10 min incubation. The results showed that H_2O_2 had excellent effect and all bacteria showed no growth on blood agar, while chlorhexidine and iodine had excellent effects on *E. coli* and *Klebsiella*. The next antiseptic in descending order of their effectiveness was Dettol since both *E. coli* and *Klebsiella* were able to survive. However, all studied bacteria showed full growth and not affected by ethanol.

DISCUSSION

This study showed that antiseptics used in PPP-UiTM still have considerable bactericidal effects on nosocomial bacteria. In 2010, Malaysia was estimated to have hundred thousand cases of nosocomial infection, amounting to 13.9% of the overall hospital admissions (Frost and Sullivan, 2011). This study revealed that different types of nosocomial bacteria vary in their response to different types of antiseptics.

Table 1. Bacterial inhibition zones by using different concentrations of antiseptics.

Antiseptic/Bacteria		Antiseptic concentrations (%)										Positive control
		100	90	80	70	60	50	40	30	20	10	
Chlorhexidine	MRSA	25	24	23	23	22	22	22	21	20	20	20[a]
	A. baumannii	20	18	17	17	17	16	16	16	15	14	15[b]
	E. coli	26	22	20	20	18	18	18	17	17	16	30[c]
	Klebsiella spp.	24	24	22	22	21	20	20	18	17	16	30[c]
	P. aeruginosa	22	21	21	21	20	19	19	18	18	17	16b
H_2O_2	MRSA	30	30	30	30	30	30	30	30	30	30	20[a]
	A. baumannii	30	30	29	29	28	28	27	27	22	20	15[b]
	E. coli	38	38	38	38	38	36	25	23	20	18	30[c]
	Klebsiella spp.	34	34	33	33	32	32	31	31	30	30	30[c]
	P. aeruginosa	30	30	28	28	26	26	25	24	20	18	15[b]
Iodine	MRSA	30	28	26	25	24	24	23	22	18	17	20[a]
	A. baumannii	21	21	20	20	19	18	15	14	12	10	15[b]
	E. coli	35	33	31	26	12	10	8	0	0	0	31[c]
	Klebsiella spp.	24	22	22	20	20	12	10	8	8	0	30[c]
	P. aeruginosa	13	12	12	11	10	9	8	7	0	0	15[b]
Ethanol	MRSA	0	0	0	0	0	0	0	0	0	0	20[a]
	A. baumannii	0	0	0	0	0	0	0	0	0	0	14[b]
	E. coli	0	0	0	0	0	0	0	0	0	0	30[c]
	Klebsiella spp.	0	0	0	0	0	0	0	0	0	0	30[c]
	P. aeruginosa	0	0	0	0	0	0	0	0	0	0	14[b]
Dettol	MRSA	30	29	28	27	26	25	24	23	22	21	20[a]
	A. baumannii	34	32	24	24	23	22	22	20	20	20	16[b]
	E. coli	20	18	18	16	16	14	14	14	14	12	30[c]
	Klebsiella spp.	20	18	18	16	15	14	14	14	13	12	30[c]
	P. aeruginosa	0	0	0	0	0	0	0	0	0	0	15[b]

a: Vancomycin, b: Polymyxin B, c: Imipenem.

Table 2. Minimal inhibitory concentration (MIC) of antiseptics against nosocomial bacteria.

Antiseptics	MRSA (%)	*A. baumannii* (%)	*E. coli* (%)	*Klebsiella spp.* (%)	*P. aeruginosa* (%)
Chlorhexidine	10	20	++	++	10
HPX	10	10	50	10	10
Iodine	30	40	80	++	++
Ethanol	++	++	++	++	++
Dettol	10	10	++	++	++

++ Full growth of bacteria seen on bijou bottles and blood agar plates.

Table 3. Antibacterial effect of highest concentration of antiseptics on nosocomial bacterial on blood agar.

Antiseptics	Bacteria				
	MRSA	*A. baumannii*	*E. coli*	*Klebsiella*	*P. aeruginosa*
Chlorhexidine	No growth	Moderate growth	No growth	No growth	No growth
Hydrogen peroxide	No growth	No growth	No growth	No growth	No growth
Iodine	No growth	No growth	No growth	Moderate growth	Moderate growth
Ethanol	Full growth	Full growth	Full growth	Full growth	Full growth
Dettol	No growth	No growth	Moderate growth	Moderate growth	Full growth

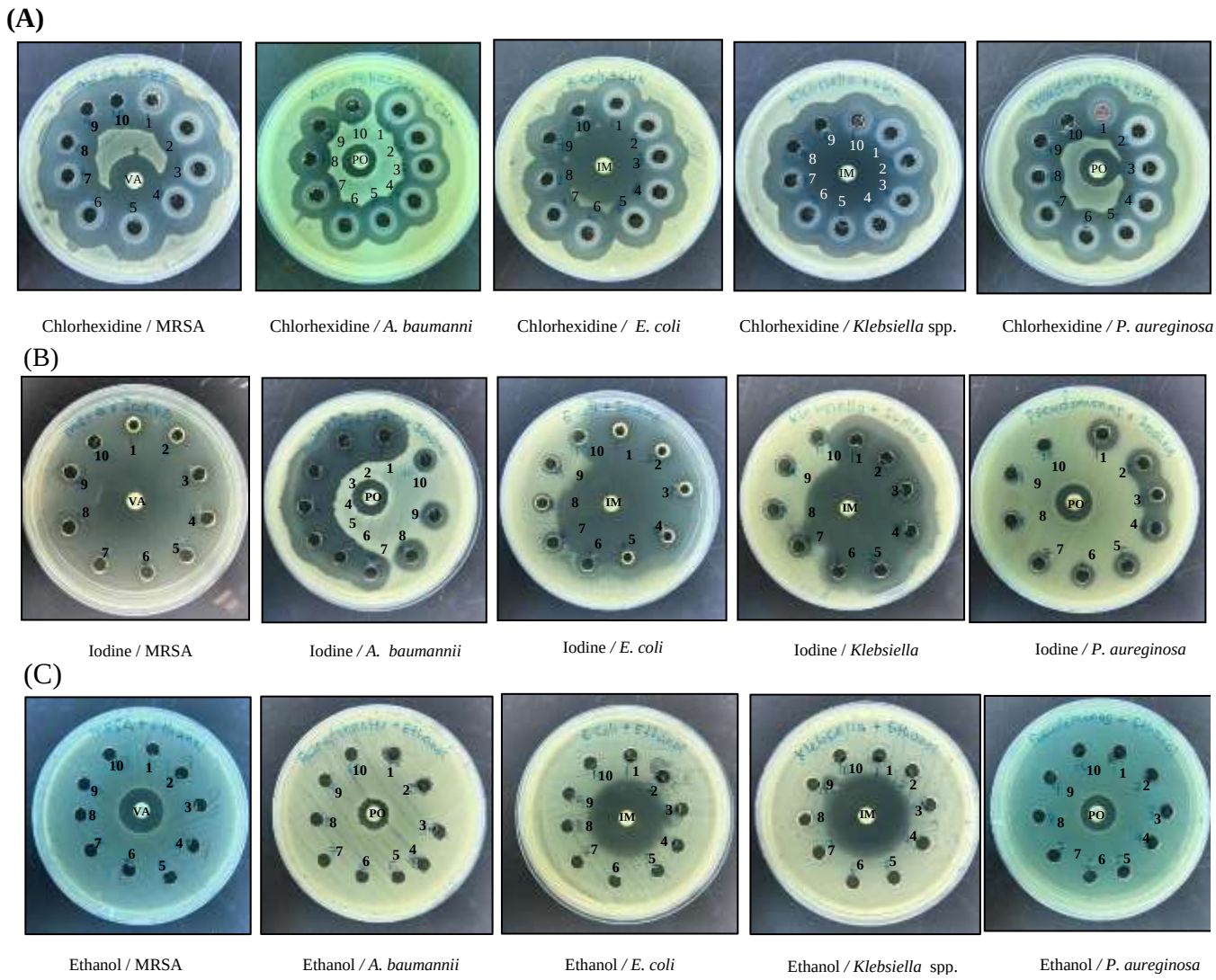

Figure 1. Susceptibility testing of A. Chlorhexidine B. Iodine and C. Ethanol against nosocomial bacteria.

Chlorhexidine is a broad spectrum bactericidal antiseptic that is widely used as in dental, surgical settings and also used in handwashing. Same as previously reported, this study showed excellent inhibitory effects of chlorhexidine against MRSA, *A. baumannii* and *P. aeruginosa* even with low concentrations (Ekizoglu et al., 2016; Lanjri et al., 2017); while on *E. coli* and *Klebsiella* it was ineffective with lesser inhibitory zones compared to Imipenem as a consequent of the outer membrane which acts as a selective permeability barrier in limiting the entry of many harmful chemical compounds into the bacterial cell (Russell et al., 1998). Chlorhexidine is working on the cytoplasmic membrane and its cationic nature helps in connection with the anionic group (phosphate groups of teichoic acids in Gram-positive bacteria and lipopolysaccharide in Gram negative bacteria) on the bacterial surface with resulting modification of membrane permeability. The effect is mainly due to electrostatic interaction of the chlorhexidine with the acid phospholipids in the cytoplasmic membrane which implies actual absorption onto the cytoplasmic membrane of Gram positive and Gram negative bacteria and leading to a destructive effect. Using chlorhexidine at low concentrations resulted in a bacteriostatic effect while at high concentrations; it can cause membrane disruption and cell death due to coagulation of the cytoplasm (Estrela et al., 2003). Chlorhexidine lasts much longer than other antiseptics; therefore, it is often combined with alcohol in a newer skin preparation composed of 2% chlorhexidine gluconate and 70% isopropyl alcohol (Mangram et al., 1999). It is reported to have a rapid onset of action and has persistent activity to reduce microbial burden on patients' skin and prevent secondary bacterial infections (Climo et al., 2013). Adaptation and resistance to chlorhexidine has been reported previously among MRSA and many other Gram-negative bacteria

including *P. aeruginosa* and *E. coli* (Kampf and Kramer, 2004).

Hydrogen peroxide has a broad-spectrum effect against bacteria, bacterial spores, viruses and yeasts (Brudzynski, 2006). This study showed excellent inhibitory effects of H_2O_2 against both Gram-positive and negative bacteria even with lowest concentration of H_2O_2 due to a potent oxidant which produce a hydroxyl radicals which in turn will attack cell membrane, lipids, DNA, and other essential cell components (Mai-Prochnow et al., 2008). The results of the present study were in agreement with previous report by Lemmen et al. (2015) who deduced that H_2O_2 was effective against nosocomial pathogens such as MRSA and multidrug-resistant *A. baumannii* in hospital settings. Previous study by Kenar et al. (2007) concluded that higher concentrations of H_2O_2 (10 to 30%) and prolonged interaction are required for sporicidal activity, unfortunately the effect of H_2O_2 on fungus was not included in this study. Although H_2O_2 showed inhibitory effect on *E. coli* after 50% dilution but it is still effective in reducing the expression of all the virulence factors of *E. coli* by oxidative stress of H_2O_2 (Hegde et al., 2008). Thus, H_2O_2 clearly shows effectiveness against all nosocomial bacteria since it has the largest zone of inhibition among all the antiseptics.

The results of this study showed that iodine had comparable effects to chlorhexidine but less than H_2O_2. These results however were not in agreement with previous finding that chlorhexidine are more effective than iodine in reducing nosocomial infections (Nishimura, 2006). The results reveal variations of the effect of Iodine on different bacteria with different dilutions. The best inhibitory effect of iodine seen against MRSA, *A. baumannii* and *E. coli* with dilutions of 30, 40 and 80%, respectively. Hence the more dilution of iodine might weaken the iodine linkage to the carrier polymer with an accompanying increase of free iodine in solution. Therefore, iodine must be diluted according to the supplier's directions to achieve antimicrobial activity. Based on the aforementioned results, we recommended to use iodine in lower concentrations to avoid skin irritation as previously reported (Murthy and Krishnamurthy, 2009). Due to its rapid, effective and broad-spectrum antimicrobial effects, povidone iodine is likely to remain a highly effective in preventing nosocomial infections in the foreseeable future. A previous clinical trials revealed that iodine was significantly superior to other antiseptic agents such as silver sulfadiazine cream and non-antiseptic dressings, but had lesser effect than rifampicin local cream. Therefore, iodine should be considered among the modern antiseptic agents. In contrast, iodine has many cellular targets, including fatty acids, nucleotides and the free sulfur amino acids cysteine and methionine in proteins 63. This makes the development of resistance unlikely.

Ethanol has a rapid broad-spectrum antimicrobial effect against bacteria, viruses and fungi; however, it is not sporicidal, therefore it is not recommended for sterilization, yet ethanol is used as antiseptics for both hard-surface and skin (McDonnell and Russell, 1999). This study demonstrates that all bacteria were resistant to ethanol at various concentrations. These results were in agreement with recent report by Pidot et al. (2018) who stated that the multidrug-resistant bacterium has become gradually tolerant to the ethanol in widely used hospital disinfectants such as hand rub solutions. Although ethanol performs a multifunctional inhibitory effect on bacterial cells, however the resistant bacteria can overcome the denaturation of proteins, inhibition of DNA, RNA, protein, and peptidoglycan synthesis by ethanol. Researchers have found out that drug-resistant bacteria that commonly cause hospital infections have the chance to develop resistance to ethanol (Cariz, 2018).

Dettol had broad spectrum activity as it inhibited the growth of Gram positive and Gram negative bacteria. Dettol is working through the penetration into the cell and action at the target site through intra-cellular mechanism. Both MRSA and *A. baumannii* were most susceptible to Dettol at different concentrations even at lowest concentration of 10%. It still showed the highest inhibition zone at 30 and 34 mm for MRSA and *A. baumannii*, respectively, however *P. aeruginosa* was resistant even with 100% concentration. Also, both *E. coli* and *Klebsiella* were resistant at 100% concentration. Previous studies showed variations for the effects of Dettol on different pathogens due to difference in the species or strains of the organisms or the techniques used (Rutala et al., 2000).

Conclusions

This study has confirmed that H_2O_2 was the strongest antiseptic against nosocomial bacteria followed by chlorhexidine, whereas ethanol was the weakest one. Determination of antimicrobial efficiency of antiseptics regularly is crucial to reduce NI which also could be reduced by using a proper antiseptic with adequate dilutions.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

In vivo safety and hypolipidemic effect of *Bifidobacterium adolenscentis* CH_2 in female albino rats

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This paper studied the safety and physiological effect of acute intake of *Bifidobacterium adolescentis* CH_2 in albino rats using yogurt as a carrier. Thirty-six female albino rats were divided into three groups: control group received standard diet and water daily; yogurt group received standard diet, water, and 0.5 ml yogurt; *B. adolenscentis*-yogurt (probiotic yogurt) group received standard diet, water, and 0.5 ml *B. adolenscentis* CH_2-yogurt, at a dose of 3.6×10^6 cfu *B. adolescentis* CH_2/animal. Yogurt and *B. adolenscentis* CH_2-yogurt administration was done daily by oral gavage for four weeks. Rats were monitored daily for feed intake and weight, and weekly for organ conditions and functions. Histology of liver and kidney were performed at week 3 and 4. There was no significant statistical difference in feed intake for the three groups. Results established no significant difference in average organ/body weight ratios of liver, lung, heart, and spleen in groups at week 4. Concentration of clinical parameters- albumin, bilirubin, aspartate aminotransferase, glucose, total protein, and urea- indicated no significant difference among groups. This study recorded lower triglycerides and total cholesterol levels in *B. adolescentis*-yogurt group in week 4. Kidney and liver histopathology confirmed that the studied *B. adolescentis* had no negative effects on rat liver and kidney. Significant reduction ($P<0.05$) in body weight gain for *B. adolescentis*-yogurt group was observed from week three. Our findings showed that *B. adolescentis* CH_2 is safe for acute intake. Results suggested a hypolipidemic and weight reduction effect on prolong usage, indicating a potential for application in weight management and cardiovascular disease control.

Key words: *Bifidobacterium adolescentis,* probiotics, *in vivo,* safety, hypolipidemic.

INTRODUCTION

Probiotics as key gut microbiome contribute functional genes that affect host physiology and health (Greenhalgh et al., 2016). Certain species of Lactic Acid Bacteria (LAB) and bifidobacteria are commonly used as probiotics because of their well-known beneficial effects to host health (Goldin and Gorbach, 1992; Oakey et al., 1995; O'Bryan et al., 2013). Studies have shown that probiotics can stimulate the immune system, decrease serum cholesterol, alleviate lactose intolerance, control infections, act as antibiotics, suppress tumors and protect

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against colon/bladder cancer (Bordoni et al., 2013; Di Gioia et al., 2014; Ejtahed et al., 2011; Isolauri et al., 2012; Scheinbach, 1998). The antiviral activity of *Bifidobacterium adolescentis* was also reported (Cha et al., 2012; Kim et al., 2014). *Bifidobacterium* species *are* among the most widely used and studied probiotic microorganisms. *Bifidobacterium* species predominance in the gut is reported to be due to their possession of large number of genes required for the degradation and use of a wide range of carbohydrates, outnumbering what is found in other gut microbes (Milani et al*.,* 2014, 2016; O'Callaghan and van Sinderen, 2016). Species of bifidobacteria considered as probiotics include: *Bifidobacterium infantis*, *B. adolescentis*, *Bifidobacterium animalis* subsp *animalis*, *Bifidobacterium animalis* subsp *lactis*, *Bifidobacterium bifidum*, *Bifidobacterium longum*, and *Bifidobacterium breve* (Fijan, 2014).

In vivo animal toxicity studies of probiotics at a dose relative to the anticipated intake provide suitable evidence of safety. Absence of observable harmful effect in acute toxicity test for a probiotic strain is considered a good indication of the possible safety of the strain for human consumption, although human studies are necessary before final application in human. In toxicological studies, abnormalities in animal weights, concentrations of blood biochemical parameters, organ/body weight ratios, and histopathology of relevant tissues are some biomarkers that underline evidence of negative impact of a consumed substance. For instance, a high level of total cholesterol and triglycerides indicate hyperlipidemia, a metabolic syndrome and risk factor in coronary heart disease (Dhingra et al., 2014; Kolovou et al., 2005).

Live microorganisms, such as probiotics, are often dosed through a carrier. Dairy products are recognized as ideal vehicle for delivering probiotic bacteria to the human gastrointestinal tract (Homayouni et al., 2012). Yogurt is one of the most popular fermented dairy products known for centuries to have universal acceptance in terms of nutritional and health benefits. It is considered as a healthy food due to its high digestibility and bioavailability of nutrients (Yadav et al., 2015). Though researchers previously reported that ingestion of some *Bifidobacterium* strains caused no observable harmful effects in animals (Chenoll et al., 2011; Mohan et al*.*, 2006; Odamaki et al., 2007; Salazar et al., 2011; Xiao et al., 2006) and European Food Safety Authority (2017) recognized *B. adolescentis* as presumptively safe, it is still needful to ensure the safety of any probiotic microorganism prior to its application in product development. Continuous surveillance is also necessary given the fact that most probiotic *in vivo* effects are strain specific and that microbial genetic change is always a possibility. The objective of this study was to evaluate the *in vivo* physiological effects of an isolated strain of *B. adolescentis* proposed to be used as a probiotic agent, in experimental rats, using yogurt as the delivery vehicle.

MATERIALS AND METHODS

Bacterial strain and growth condition

B. adolescentis strain, designated *B. adolescentis* CH_2, was isolated from chicken (*Gallus gallus domesticus*). Its identification and probiotic properties investigations were previously reported (Onyibe et al., 2013a, b). *B. adolescentis* CH_2 was cultivated by growing in De Man Rogosa and Sharpe (MRS) medium (Oxoid) adjusted to pH 5.8 – 6.4 and supplemented with 0.05% L-cysteine hydrochloride. Culture was incubated anaerobically at 37°C for 48 h in Oxoid anaerobic jar system with Oxoid AnaeroGen: O_2 below 1%, and CO_2 between 9 - 13%.

Yoghurt and fortified (probiotic) yoghurt production

Production of yogurt and *B. adolescentis* CH_2-yogurt (fortified yogurt/probiotic yogurt) was by the modified method of Heller (2001). Two hundred and ten grams (210 g) of milk powder and 40 g of granulated sugar were dissolved in 1-L sterile distilled water, pasteurized at 95°C for 10 min, cooled to 44°C and inoculated with yogurt starter culture - *S. thermophilus, L. bulgaricus* and *L. acidophilus* (Yogomet, Germany). Cooled pasteurized inoculated milk was distributed into 500-mL flasks after thorough mixing with magnetic stirrer. Flasks and contents were incubated in a water bath at 42-43°C. The pH was monitored until pH dropped to 4.5. Produced yogurt was pasteurized at 85°C for 15 min and refrigerated overnight. Pasteurized yogurt was subsequently divided into two and one portion was inoculated with *B. adolescentis* CH_2 culture as an adjunct to produce the *B. adolescentis* CH_2-yogurt (fortified yogurt/probiotic yogurt). Samples were stored refrigerated prior to use.

In vivo safety and physiological effect study in rats

Thirty-six two weeks old female albino rats were obtained from the Animal Facility of the College of Medicine, University of Lagos Teaching Hospital, Lagos, Nigeria. Animal handling procedure was in accordance with the U.S government principles for the utilization and care of vertebrate animals in testing, research, and training (National Academy of Sciences, 2011). The animals were maintained and housed in cages at room temperature (28 ± 3°C). They were fed with standard rodent diet and water *ad libitum,* and allowed to acclimatize for 2 weeks. After acclimatization, rats were randomly divided into three groups of 12 rats: group one (control) were given standard diet and water daily; group two (yogurt group) were given standard diet, water and daily oral dose of 0.5 ml yogurt; while group three (*B. adolenscentis*-yogurt/probiotic yogurt group) were given standard diet, water, and daily oral dose of 0.5 ml yogurt containing approximately 7.2×10^6 cfu/ml *B. adolenscentis,* representing an intake of about 3.6×10^6 cfu *B. adolescentis* per animal per day. This administered intake was based on the recommended viability of ≥ 10^6 cfu probiotic culture. Applying the uncertainty factor of 100-fold (10-fold corrections for species differences and 10-fold corrections for human variability), this amounted to an intake of 2.48×10^5/kg body weight/day which is equivalent to 1.74×10^7 per day for an average 70 kg human. The *B. adolenscentis* dosage was not adjusted for body weight throughout the study period. Yogurt and *B. adolenscentis* fortified yogurt were supplied biweekly to ensure freshness and culture viability. All animals in each cage were administered the same dose daily by the orogastric feeding tube for four weeks.

Rats were monitored daily for weight and physical appearance throughout the study. Body weight and feed intake were recorded daily. *B. adolescentis* CH_2 acute toxicity was assessed by evaluating the rat development and organs, and by analyzing blood

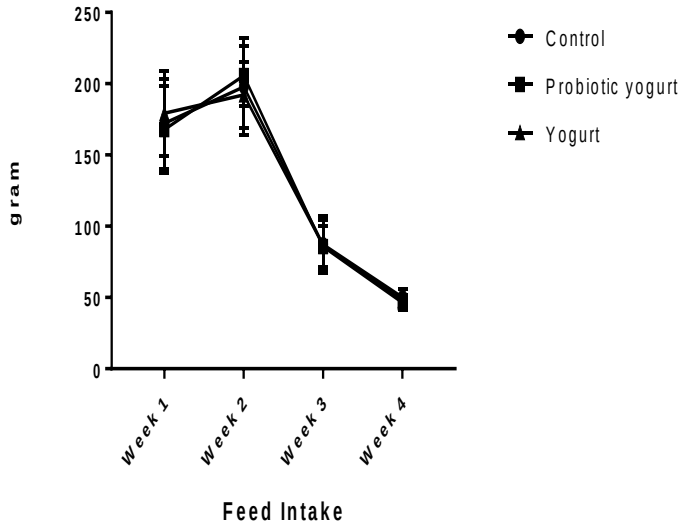

Figure 1. Average feed intake.

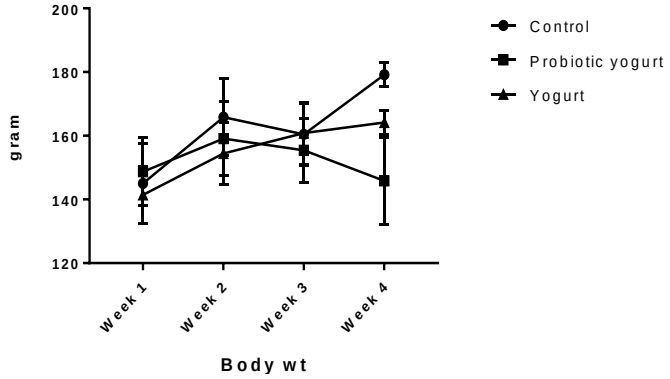

Figure 2. Average body weight.

concentrations of some relevant biochemical compounds. The results obtained for the treated (*B. adolescentis*-yogurt) group were compared with results for the control and yogurt groups. For evaluation of organs conditions and functions, three rats from each group were sacrificed weekly by cervical dislocation under anesthesia. Their blood samples were collected, transferred into lithium heparin bottle, and centrifuged for 15 min to obtain plasma. Plasma concentrations of glucose, albumin, bilirubin, total protein, total cholesterol, high density lipoprotein-cholesterol, triglycerides, urea, and creatinine were determined using BIOLABO reagent kits, while Randox reagent kit was used for aspartate aminotransferase determination. Assays were carried out following the commercial kits' instructions. The weight of organ was recorded weekly.

Histopathology

Histopathological evaluation of dissected rat's kidney and liver were performed at week 3 and 4. The liver and kidney tissues were processed and embedded in paraffin. Sections were cut, stained using hematoxylin and eosin method, and subsequently viewed under the microscope.

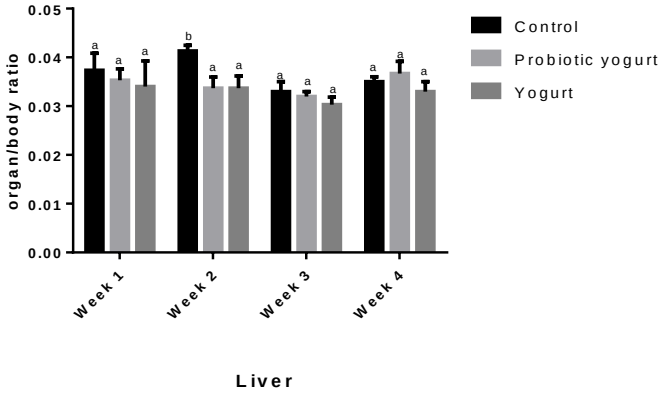

Figure 3. Average Liver-Body weight ratio. Bars with different letters in the same week are significantly different (P<0.05).

Statistical analysis

Data obtained were statistically analyzed by one-way ANOVA with multiple comparisons using graph pad prism 6.0. A P value less than 0.05 (P<0.05) was considered significant.

RESULTS

No incidence of animal mortality was recorded in any group. There was no significant difference in feed intake among the groups throughout the period of investigation (Figure 1). Results indicate no statistical significant difference in rats development and body weight in all groups up to day 15 of treatment. There was a reduction (P<0.05) in body weight gain of the *B. adolescentis*-yogurt (probiotic yogurt) group from week 3 of administration, while the body weights of the control and yogurt groups were increased (Figure 2).

There was no significant difference (P>0.05) in the average organ/body ratios of the liver, heart, lung, and spleen between the *B. adolescentis*-yogurt and yogurt groups, and with each group compared with the control at week 4 (Figures 3 to 6). Evidence obtained from results inferred that there was no significant difference (P>0.05) in the average organ/body weight ratio of the lung and liver of the *B. adolescentis*-yogurt and yogurt groups compared with the control group at week 1, 3, and 4 of administration. The average lung/body weight ratio and liver/body weight ratio for *B. adolescentis*-yogurt and yogurt groups indicated no significant difference over the four weeks including week 2 of intake. However, results of the average liver/body and lung/body weight ratios of the control group at week 2 compared to the yogurt and *B. adolescentis*-yogurt groups showed significant difference.

Figure 7 represent the average brain/body weight ration. When compared with the control group, a significant difference was detected in the average brain/body weight ratio (P<0.05) of the *B. adolescentis*-

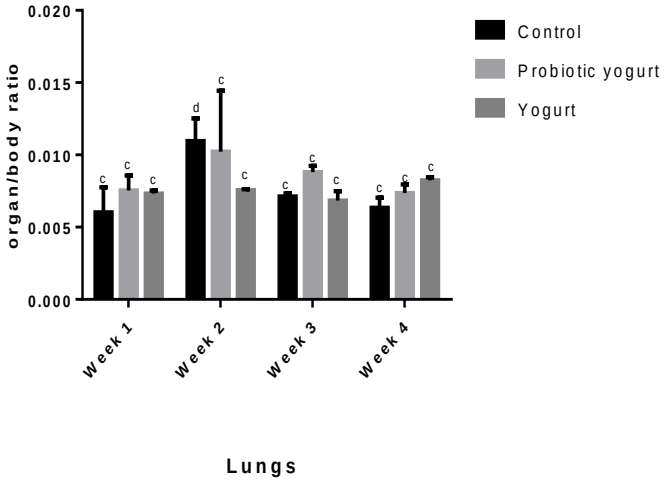

Figure 4. Average Lung-Body weight ratio. Bars with different letters in the same week are significantly different (P<0.05).

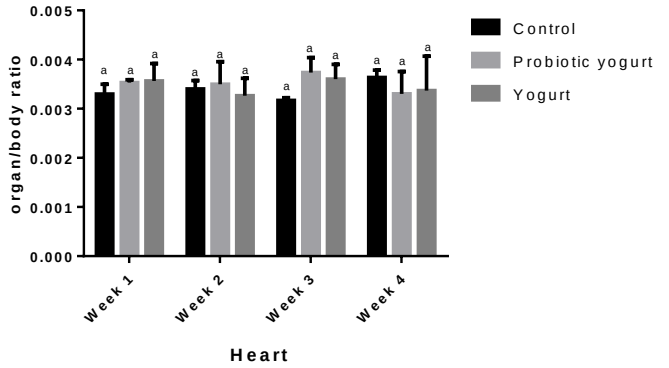

Figure 5. Average Heart-Body weight ratio. Bars with different letters in the same week are significantly different (P<0.05).

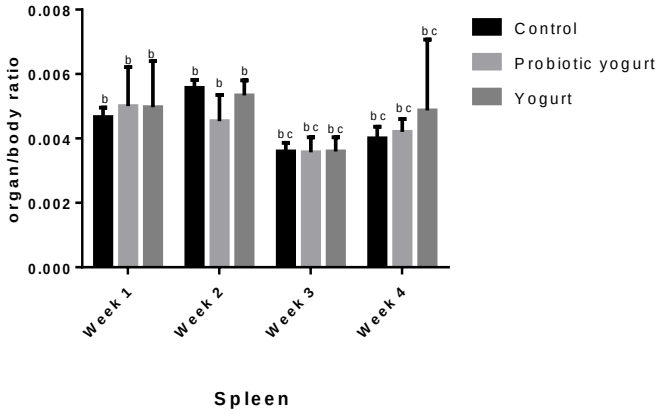

Figure 6. Average Spleen-Body weight ratio. Bars with different letters in the same week are significantly different (P<0.05).

yogurt and the yogurt group throughout the four weeks of administration except at week 2. We observed no

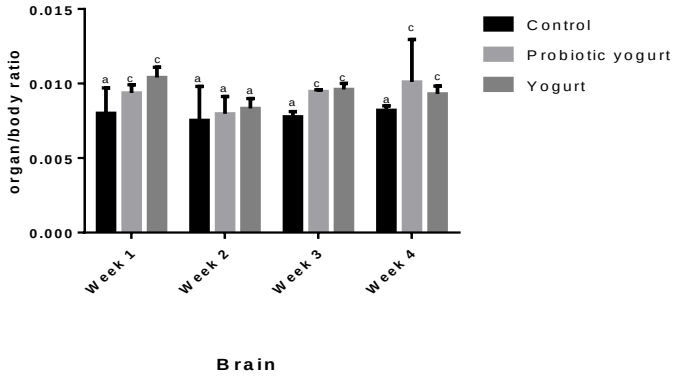

Figure 7. Average Brain-Body weight ratio. Bars with different letters in the same week are significantly different (P<0.05).

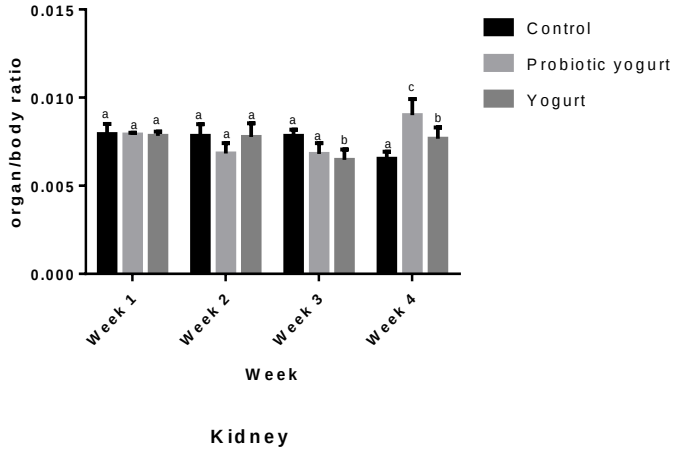

Figure 8. Average Kidney-body weight ratio. Bars with different letters in the same week are significantly different (P<0.05).

significant difference in the brain/body weight ratio between the *B. adolescentis*-yogurt and the yogurt group from weeks 1 to 4. However, the organ/body weight ratio of the brain for the *B. adolescentis*-yogurt and the yogurt group were significantly higher (P<0.05) compared to the control group in weeks 1, 3, and 4 of investigation. The brain/body weight ratio at week 4 was 0.0082, 0.0101, and 0.0093 for the control, *B. adolescentis*-yogurt, and yogurt group respectively. There was no significant difference (P≥0.05) in the organ/body weight ratio values of the kidney in the three groups from week 1-2. However, values were significantly higher (P=0.001) in the yogurt and probiotic yogurt group in week 4 (Figure 8).

As illustrated in Figures 9 to 14, there were no significant statistical differences (P>0.05) in the average blood concentrations of Aspartate aminotransferase (AST), Glucose, Total protein, Albumin, Bilirubin, and Urea between the *B. adolescentis*-yogurt and yogurt groups as well as with each group compared to the control group throughout the study period. The blood plasma level of triglycerides was lower for the *B.*

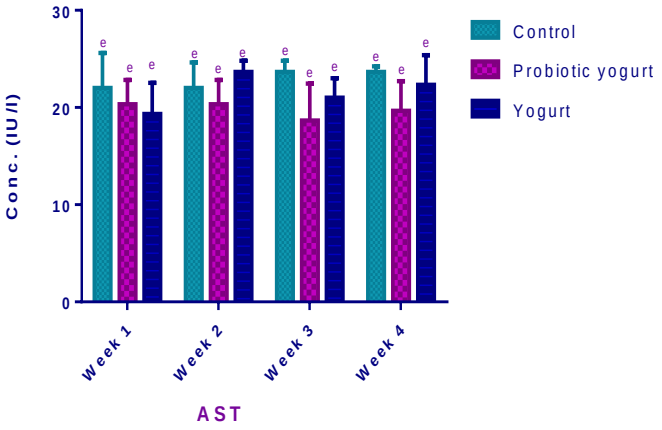

Figure 9. Average aspartate aminotransferase concentration. Bars with different letters in the same week are significantly different (P<0.05).

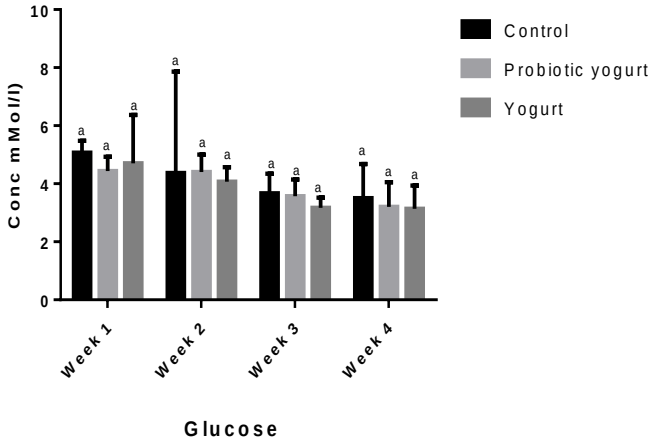

Figure 10. Average concentration of glucose. Bars with different letters in the same week are significantly different (P<0.05).

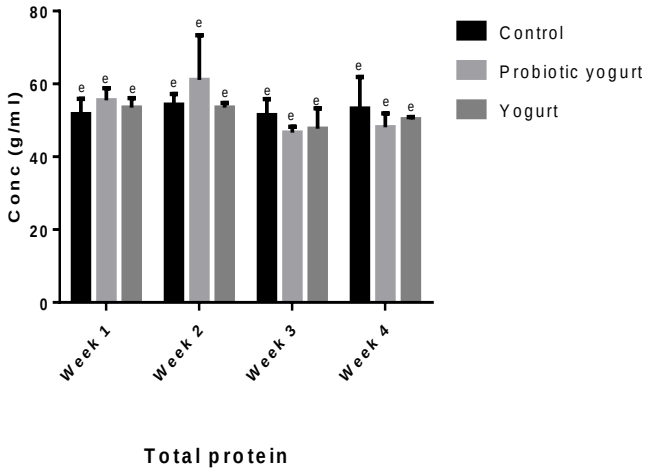

Figure 11. Average concentration of total protein. Bars with different letters in the same week are significantly different (P<0.05).

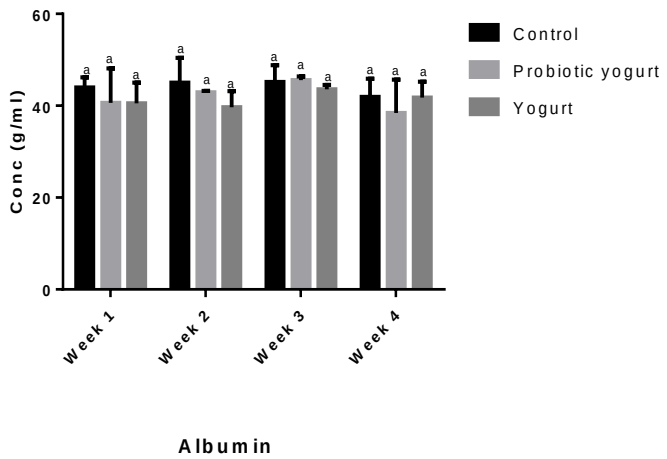

Figure 12. Average albumin concentration. Bars with different letters in the same week are significantly different (P<0.05).

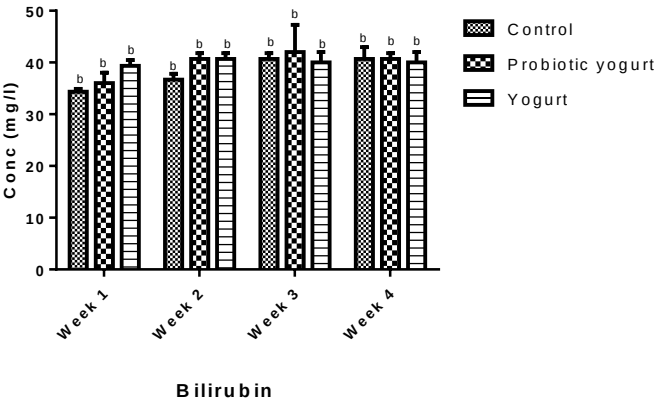

Figure 13. Average bilirubin concentration. Bars with different letters in the same week are significantly different (P<0.05).

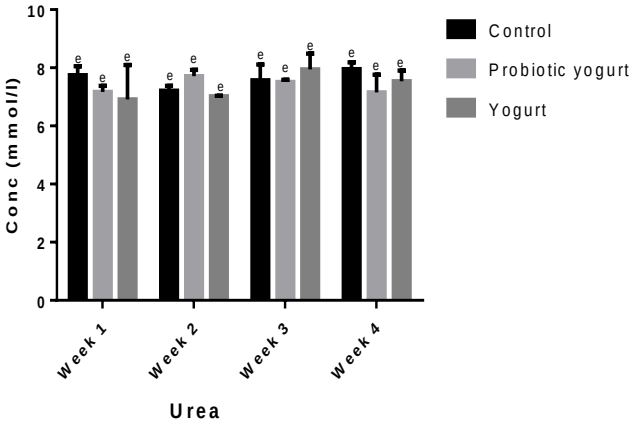

Figure 14. Average Urea concentration. Bars with different letters in the same week are significantly different (P<0.05).

adolescentis-yogurt group than for the control and yogurt

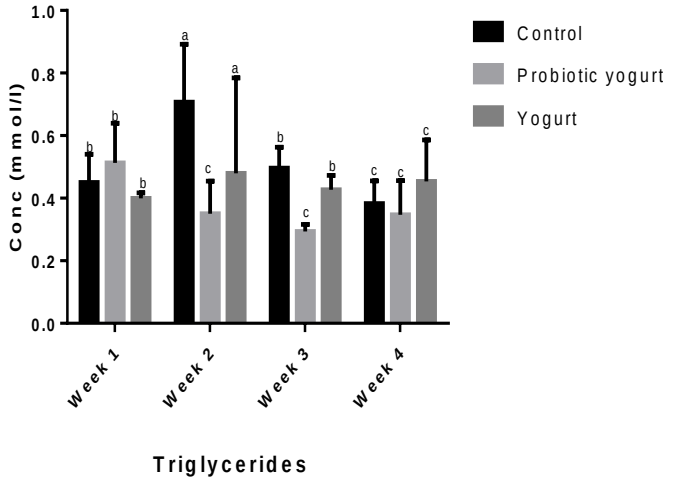

Figure 15. Average concentration of triglyceride. Bars with different letters in the same week are significantly different (P<0.05).

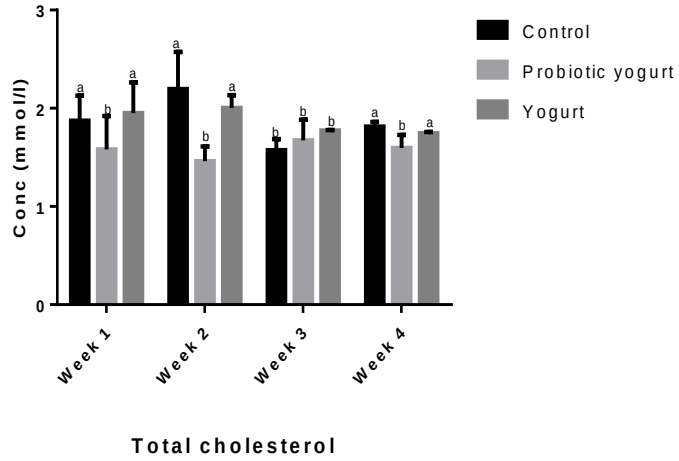

Figure 16. Average total cholesterol concentration. Bars with different letters in the same week are significantly different (P<0.05).

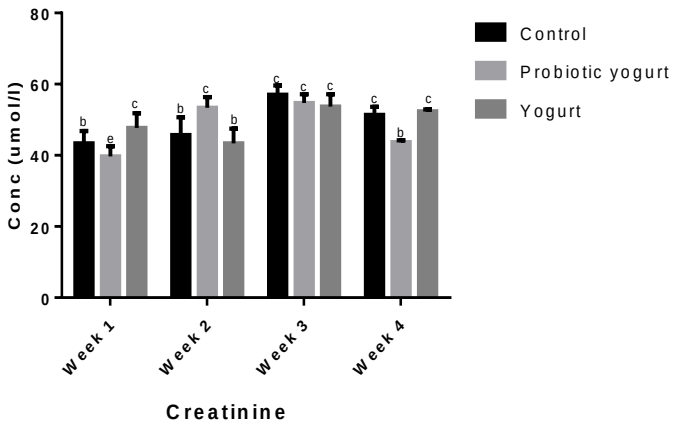

Figure 17. Average creatinine concentration. Bars with different letters are significantly different (P<0.05).

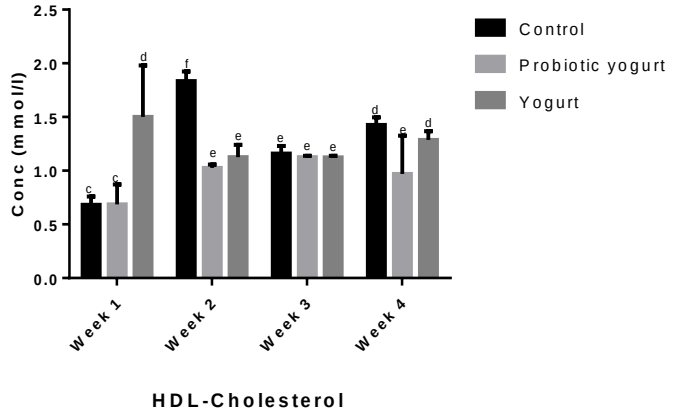

Figure 18. Average High-density lipoprotein-cholesterol concentration. Bars with different letters are significantly different (P<0.05).

groups at week 2, 3, and 4 (Figure 15). The value for the average concentration of total cholesterol in *B. adolescentis*-yogurt group was lower compared to the control group and yogurt group by week 4 of administration (Figure 16).

Following the four weeks intake of *B. adolescentis* CH_2-yogurt, the blood concentration of creatinine for the *B. adolescentis*-yogurt group was significantly lower (P=0.0001) compared to the control group and yogurt group by week 4 as shown in Figure 17. The average high-density lipoprotein-cholesterol concentration in the *B. adolescentis*-yogurt animals did not reveal a significant difference when compared against its values in the control group at week 1 and 3, and with yogurt group at week 2 and 3. By week 4 of the investigation, the concentration was reduced in *B. adolescentis*-yogurt animals when compared with the control and yogurt animals (Figure 18).

As presented in Figures 19 and 20, the administered *B. adolescentis* CH_2-yogurt did not result in any abnormalities in animal kidney or liver. The histopathological examination of kidney for all groups did not detect any abnormalities (Figures 19). The liver histological section for the control and *B. adolescentis*-yogurt did not reveal any difference between the tested group (*B. adolescentis*-yogurt) and the control group, while the liver histopathological examination for yogurt group showed sinusoidal congestion (Figure 20).

DISCUSSION

B. adolescentis enjoy the GRAS (Generally Regarded As Safe) and presumptively safe species for human and animal consumption status (FAO/WHO, 2006; European Food Safety Authority, 2017). Past researches have also

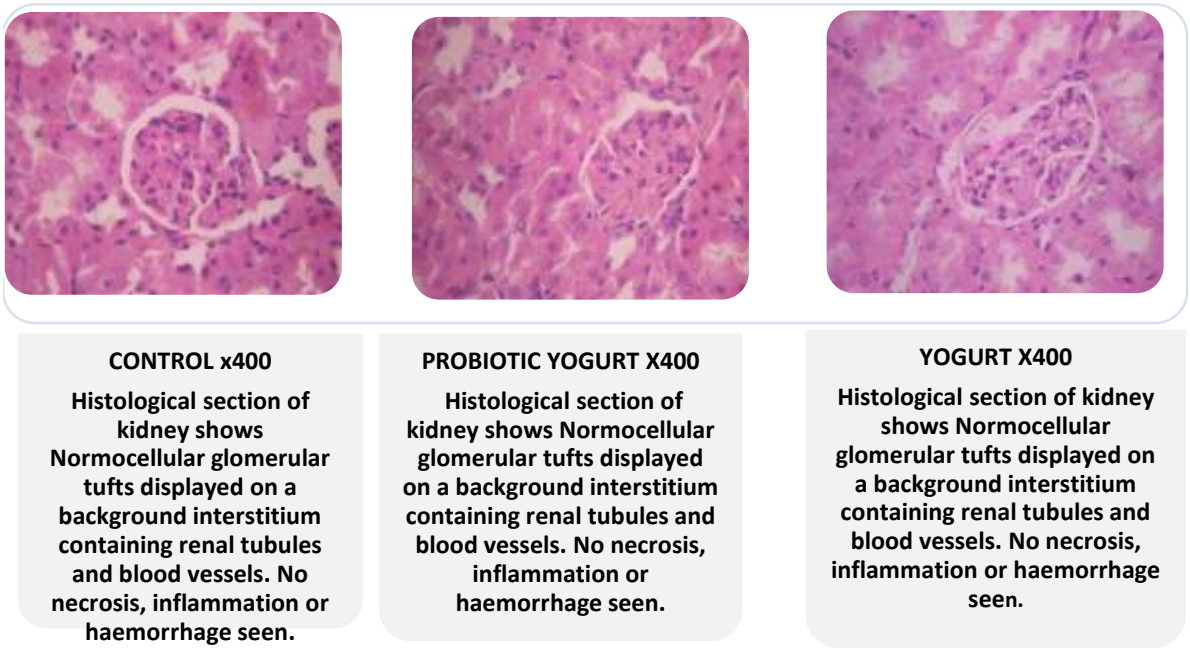

Figure 19. Histological section of kidney.

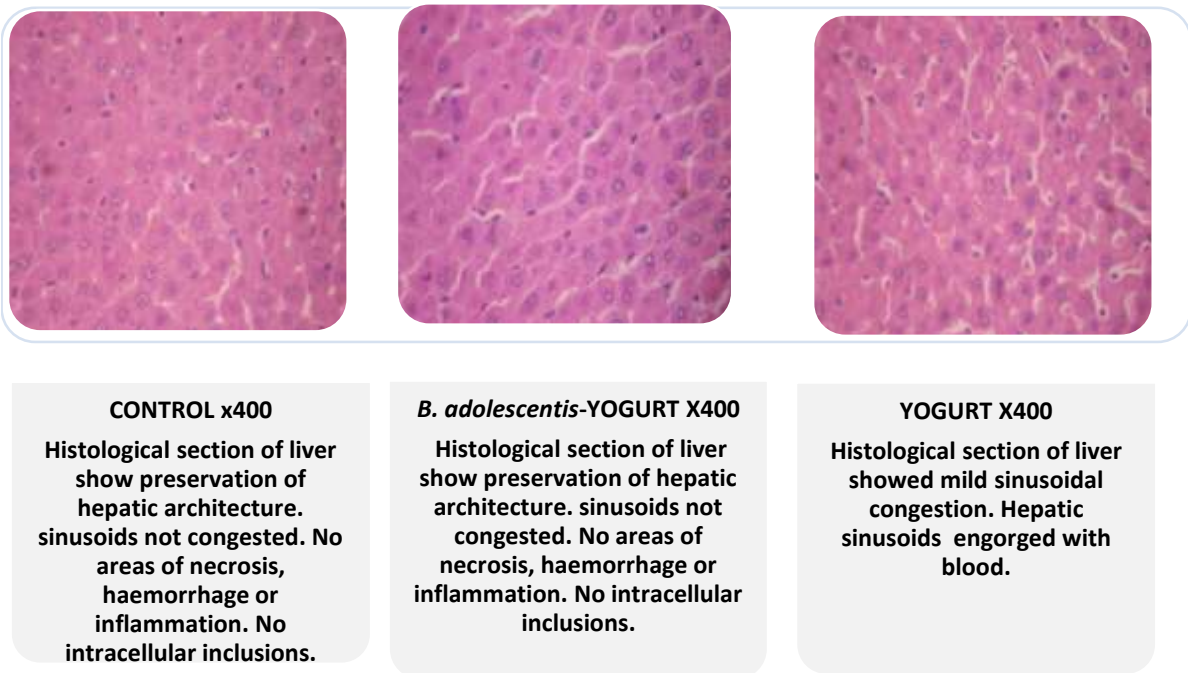

Figure 20. Histological section of liver.

documented the safety of *B. adolescentis and other Bifidobacteriun* species (Munoz et al., 2011; Patole et al., 2016; Salazar et al., 2011). Despite the *B. adolescentis* safe for consumption status, it is important that new isolates be confirmed safe before application as probiotic agents in animals and human. Some microbial characteristics or effects are strain specific. Moreover, continuous surveillance of probiotic microorganisms is necessary to continuously guarantee consumer safety. In this *in vivo* study we evaluated the effect of *B. adolescentis* CH_2 dose on female albino rats by comparing the results obtained for *B. adolescentis*-yogurt

group with that obtained for the yoghurt and control groups.

Effect of *Bifidobacterium adolescentis* CH_2 on feed intake and body weight

There was no statistical significant difference in all groups for feed intake rate throughout the study period. Results up to week 2 (day 15) of investigation showed comparable growth for all groups. At week 3 and 4, despite the evidence of no significant difference in feed intake for all groups during the investigation period of four weeks, there was a significant reduction in average body weight for the *B. adolescentis*-yogurt group when compared with the average body weight of the control group. While the control and yoghurt group average weights increased from 145 g (week 1) to 179.167 g (week 4) and 141.458 g (week 1) to 164.167 g (week 4) respectively, the *B. adolescentis*-yogurt group average weight increased from 148.958 g (week 1) to 159.167 g (week 2) and subsequently decreased to145.833 g at week 4. This is an indication that while acute intake of *B. adolescentis* CH_2 at the applied dose of 2.48 x 10^5/kg body weight/day did not affect animal weight, chronic (prolonged) intake may lead to weight reduction. An et al. (2011), in a study with male Sprague Dawley rats, also reported lower body weight in group fed high fat diet (HFD) and bifidobactia than the group fed HFD without bifidobacteria, even though the two groups had no significant difference in caloric consumption values. They concluded that the *Bifidobacterium* species (*B. pseudocatenulatum* SPM 1204, *B. longum* SPM 1205, and *B. longum* SPM 1207) possessed antiobesity potentials. *B. adolescentis* ability to reduce body weight was previously documented (Chen et al., 2012; Lim and Kim, 2017).

Effect of *Bifidobacterium adolescentis* CH_2 on organ-body weight ratio

An important requirement in toxicological experiments is the evaluation of the effects of a substance on specific organs. Effect on organ weight and the ratio of the organ weight to body weight in toxicology studies is an important indicator for identification of potentially harmful effects of a substance. This investigation did not establish any important significant difference in the organ-body ratio of the liver, lung, heart, and spleen of the *B. adolescentis*-yogurt and yogurt groups, as well as the *B. adolescentis*-yogurt group compared with the control group. This is an indication that the *B. adolescentis* CH_2 fortified yogurt exerted no toxicology effect on these organs of the albino rats.

There was no significant difference in the average brain-body ratio between the *B. adolescentis*-yogurt and the yogurt group throughout the four weeks of study. However, the average organ/body ratio of the brain of the *B. adolescentis*-yogurt and the yogurt groups were higher compared to the control group over the 4 weeks of administration (Figure 7), indicating that this is not attributable to the administered *B. adolescentis* CH_2 strain, since the recorded difference also occurred in the yogurt group. The differences in the brain-body weight ratios of the *B. adolescentis*-yogurt and yogurt groups when compared with the control are likely results of the lower body weights (Figure 2) of the *B. adolescentis*-yogurt and yogurt group animals. Differences in body weights occur and affect organ-body ratios. Long et al. (1998) reported that brain weight has low variability compared with other organs which may impact on brain-body ratio.

There was no significant difference ($P \geq 0.05$) in the organ/body ratio value of the kidney in the three groups from week 1-2, but the value increased in the *B. adolescentis* CH_2-yogurt and yogurt groups in week 4. At the end of four weeks administration of yogurt and the *B. adolescentis* CH_2-yogurt, the average kidney/body weight rations differed among the groups. The average kidney/body weight ration for the *B. adolescentis* CH_2-yogurt group increased from 0.0079 at week 1 to 0.0090 at week 4, while the control and yogurt groups decreased from 0.0079 to 0.0065 and 0.0078 to 0.0077 respectively. However, the kidney histology of the *B. adolescentis* CH_2-yogurt group at week 3 and 4 revealed no abnormality. The significant increase in *B. adolescentis*-yogurt rats' kidney/body weight ratio in week 4 is likely due to the low body weight of the animals in the *B. adolescentis*-yogurt group during the period. Effect of variations in results of organ weights and organ-body weight ratios have previously been reported (Bailey et al., 2004; Long et al., 1998; Piao et al., 2013).

Effect of *Bifidobacterium adolescentis* CH_2 on rat kidney and liver histology

The histopathological analysis of kidney for all groups at week 3 (Figure 19) and week 4 (result not shown) were normal, indicating absence of any negative effect of treatment. There was no necrosis, inflammation or haemorrhage in the control, *B. adolescentis*-yogurt, and yogurt groups.

Histological section of liver for the control and *B. adolescentis*-yogurt groups at week 3 (Figure 20) and week 4 (result not shown) revealed preservation of hepatic architecture. There were no intracellular inclusions, sinusoids congestion, haemorrhage or inflammation, and no areas of necrosis. However, the analyzed histological sections for animals in the yogurt group showed mild sinusoidal congestion at week 3 (Figure 20) and steatosis, revealing hepatocytes with intracellular accumulations of large clear fat vacuoles at

week 4 (result not shown). This observed difference in the liver of the yogurt group compared to the control group may have resulted from the daily consumption of yogurt by the animals. The absence of this same effect in the *B. adolescentis*-yogurt group, even though animals in this group received the same daily intake of yogurt as those in the yogurt group, may be due to a hypolipidemic effect of the *B. adolescentis* CH_2 strain, indicating that the probiotic strain repressed this probable yogurt effect and ameliorated the fatty liver symptom. *B. adolescentis* CGMCC 15058 was reported to relieve increased serum and liver alanine aminotransferase and lipopolysaccharide-binding protein in rat in an induced acute liver injury condition (Li et al., 2019). According to Li et al. (2019), their studied *B. adolescentis* CGMCC 15058 protected the induced rats against liver damage and failure.

The results of kidney and liver histopathology confirmed that the four weeks *B. adolescentis* CH_2-yogurt consumption had no harmful effects on the albino female rats liver and kidney.

Effect of *Bifidobacterium adolescentis* CH_2 on rat blood biochemistry

Exposure of an animal to toxicity reflects in the blood biochemistry which usually results from effect of the substance on the organ and its functions. Authors agree that high level of some blood biochemical biomarkers can indicate metabolic organ failure and inefficient performance due to inflammation, infection, damage, or injury (Lee, 2011; Kumar et al., 2013). The results of clinical parameters for liver function tested- Albumin, Bilirubin, Aspartate aminotransferase (AST), Total protein- showed no significance difference in concentrations between the experimental groups. This showed that the administered *B. adolescentis* CH_2-yogurt did not alter the functionality of the liver of the experimental animals. The average blood concentrations of glucose, total protein, and urea of rats in the *B. adolescentis*-yogurt and yogurt groups compared favorably with the control group.

Creatinine and urea levels in the blood of animals are kidney functions tests. Creatinine, a waste product from normal muscle metabolism is easily filtered out of the blood by well-functioning kidneys which stabilize its concentration in the bloodstream.

High blood levels of urea or creatinine is an indication of malfunctioning kidney and/or kidney failure or damage in animals. The results of creatinine and urea analysis indicated that the functionality of the kidney in the experimental rats was not affected negatively by the *B. adolescentis* CH_2-yogurt. While the urea values did not vary significantly in all groups over the four weeks of evaluation, the creatinine level in the *B. adolescentis*-yogurt group was significantly lower (P=0.0001) compared to the control group and yogurt group by week 4 of administration (Figure 17).

Authors previously documented triglyceride, cholesterol, and lipid lowering activities of some *Bifidobacterium* species and strains (An et al., 2011; Tsai et al., 2014). Lim and Kim (2017) noted that oral administration of B. adolescentis IM38 lowered high fat diet-induced lipopolysaccharide levels in blood and colonic fluid of mice, with subsequent inhibition of body and epididymal fat weight gain. In an attempt to correct the lipoprotein imbalance found in the blood of children with dyslipidemia, Guardamagna et al. (2014) examined the effects of a probiotic which contained three *Bifidobacterium* strains. Their results established a decrease in total cholesterol and low-density lipoprotein cholesterol. Experiments using mice also confirmed the ability of *Bifidobacterium breve* B-3 in administered skim milk to reduce the accumulation of epididymal fat and improve total cholesterol level (Kondo et al., 2010).

This study established lower average concentrations of triglycerides and total cholesterol in rats fed with *B. adolescentis* CH_2-yogurt compared with the control group at week 4 of the study.

The blood concentration of triglycerides decreased from 0.51 mmol/L in week 1 to 0.35 mmol/L by week 4 in *B. adolescentis*-yogurt group, slightly decreased from 0.45 to 0.38 mmol/L in control group, while it slightly increased in yogurt group from 0.40 to 0.45 mmol/L. At week 4 the average blood content of total cholesterol (mmol/L**)** in control group was 1.81, *B. adolescentis*-yogurt group 1.60, and yogurt group 1.75. The hypolipidemic effect potential of *B. adolescentis* CH_2 will be investigated further to explore its possible use as a biotherapeutic.

Furthermore, animals in the *B. adolescentis* CH_2-yogurt treatment group recorded lower average weight compared to the other groups (Figure 2), showing that *B. adolescentis* CH_2 or *B. adolescentis* CH_2-yogurt could be an agent for weight management.

Conclusion

The study findings suggest that *B. adolescentis* CH_2 is a toxicologically safe probiotic for acute intake. Short-term consumption of *B. adolescentis* CH_2 supplemented yogurt revealed no undesirable effect. A hypolipidemic activity and weight reduction effect was observed at week four of daily consumption of *B. adolescentis* CH_2, suggesting that *B. adolescentis* CH_2 may have a potential for application in weight management and cardiovascular disease control.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Survey of bacterial contamination and antibiotic resistance pattern of Bangladeshi paper currency notes in Mymensingh city

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Paper currency notes, which are transferred from one individual to another, are known to carry microorganisms on their surface. As people have to exchange currencies repeatedly to buy goods and services in their everyday life, risk of spreading infectious diseases may be enhanced. Thus, it is important to identify the bacteria associated with currency and evaluate their multidrug resistance pattern. Consequently, the following study was conducted to determine some common bacterial load and their antibiotic resistance pattern of Bangladeshi paper currency notes circulating in Mymensingh city. Forty paper currency notes, comprising eight denominations from five occupational groups (Fish seller, meat seller, egg seller, vegetable seller and grocer), were collected from Mymensingh city, Bangladesh and subjected to bacteriological analysis. Total viable count, total *Staphylococcus* spp., total *Salmonella* spp. and total *Escherichia coli* counts were calculated, ranging from log 7.48±0.50 to 8.48±0.60 log cfu/paper currency (pc), 5.58±0.42 to 6.10±0.58 log cfu/pc, 5.36 to 5.88±0.38 log cfu/pc and 5.40±0.20 to 5.84±0.20 log cfu/pc, respectively from all denomination paper currency notes. Among the tested notes, 85.83% were found to be contaminated with three different bacterial isolates. Among them, *Staphylococcus* spp. were found more frequent (95%) followed by *E. coli* (87.5%) and *Salmonella* spp. (75%). Furthermore, isolated bacteria were subjected to antimicrobial susceptibility test against 8 commonly used antibiotics. The entire microorganisms tested were found resistant to Amoxicillin, Ampicillin and Ciprofloxacin, but were susceptible to Azithromycin and Norfloxacin. Thus, the present study revealed that most currency notes are contaminated with different common bacteria, including antibiotic resistant ones, and this might pose a severe public health risk.

Key words: Paper currency notes, microbial load, antibiotic resistance, Bangladesh.

INTRODUCTION

Paper currency is widely utilized as a medium of exchange all through the world for trading (Sadawarte et al., 2014). In regular transaction, paper currency is being handled by different categories of individual with the

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unhygienic condition and in this way, polluted with different kinds of pathogenic microorganisms (Borah et al., 2012). The crude materials that are utilized for making paper currency assume a huge role in harboring microorganisms. As indicated by El-dars and Hassan (2005), the blend of cotton and linen used for making paper currency offers surface area for microorganisms to grow. Previous study demonstrated that lower denomination notes get higher microbial contamination since they stay longer in circulation and exchanged more frequently (Khalil et al., 2014).

Paper currencies are normally defiled by various ways like sneezing, coughing, contacting with tainted hands or materials and placement on grimy place like pockets, socks, shoes and under floor covering (Oyero and Emikpe, 2007). In some fish, poultry and vegetable markets, the salesperson handle cash and their particular sales item simultaneously evading hand washing between their works. This practice enhances the danger of cross-contamination of microorganisms between vendors and purchasers (Michaels, 2002). Microorganisms are frequently transmitted through water, air, food and fomites (Barolia et al., 2011). Paper currency contaminated by organisms might act as fomites, plays a significant role in the transmission of microorganisms and are therefore responsible for spreading communicable diseases (Sharma and Dhanashree, 2011). Paper currency notes, thus presents specific hazard to public health, as contagious diseases can spread through this paper currency (Lalonde, 2007). Most of the paper currency are imbued with disinfectants to restrain the growth of microorganisms; yet, just a few pathogens are isolated from paper currency notes as it persists in circulation for a long time (Hanash et al., 2015). Different pathogenic microorganisms that harbor in paper currency which are related to gastroenteritis, throat disease, pneumonia, urino-genital tract contamination, peptic ulcers and lung abscess have been accounted from various place of the world (El-dars and Hassan, 2005; Hosen et al., 2006).

A few investigations in different parts of the world have been conducted and the outcome uncovered high rate of microbial contamination of paper currency notes in circulation. *Escherichia coli, Salmonella* spp., *Enteroccci* spp., *Klebsiella* spp., *Shigella* spp., *Mycobacterium tuberculosis, Vibrio cholera, Bacillus* spp., *Staphylococcus* spp., *Pseudomonas* spp., *and Corynebacterium* spp. were isolated and identified from the currency notes in different previously conducted studies (Moosavy et al., 2013; Akond et al., 2015; Boidya et al., 2015; Hanash et al., 2015; Firoozeh et al., 2017). Some of fungal species were also recovered from paper currency notes such as *Aspergillus* spp., *Rhizopus* spp., *Penicillium* spp. etc. (Barro et al., 2006; Shahram et al., 2009).

Antimicrobial resistance (AMR) is now considered as one of the most serious global threats to human health as evidenced by the WHO's report on antimicrobial resistance (WHO, 2018). Recently, multidrug resistance capabilities of microorganisms have turned out to be a major worry to public wellbeing and numerous infections have become harder to treat (Uddin et al., 2013). According to previous studies (Akond et al., 2015; Mukharjee et al., 2017) various microorganisms isolated from paper money showed drug resistance to normally used antibiotics. Firoozeh et al. (2017) found high resistance rates of *Staphylococcus* spp. and *Enterococci* spp. against tetracycline, ampicillin and erythromycin isolated from paper currency.

All class of individuals, including children habitually handles paper currencies and therefore the contaminated notes play a significant role in spreading of diseases. Most of the people in rural and urban region take food without washing their hands after handling money and saliva is used to count the money, increasing the chance of getting infection (Hosen et al., 2006). In Bangladesh, majority of people do not appropriately wash hand and are ignorant of various transmissible diseases caused by pathogenic microorganisms in currency handling (Akond et al., 2015). Just as there are a few reports on the microbial contamination of paper currency in the world, reports on Bangladesh are also scanty (Hosen et al., 2006; Ahmed et al., 2010). Considering the above stated facts, this study was conducted to investigate the presence of some common bacteria and their antibiotic resistance pattern in Bangladeshi paper currency notes, circulating in Mymensingh city.

MATERIALS AND METHODS

Collection of paper currency notes

Forty old (40) Bangladeshi paper currency notes, eight denominations including BDT (Bangladesh Taka) 2, BDT 5, BDT 10, BDT 20, BDT 50, BDT 100, BDT 500 and BDT 1000 were collected randomly from five different occupational groups including vegetable seller, meat seller, fish seller, egg seller and grocer at Kamal-Ranjit (KR) market, Bangladesh Agricultural University (BAU), Mymensingh from June to December 2017. Individual occupational groups were convinced with satisfactory explanation about the significance of the study to exchange the currency. To ensure aseptic collection, individuals were requested to drop the cash into the sterile zipper bag and were compensated with the same currency collected. The bags were immediately transported to the Microbiology laboratory of the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh for bacteriological analysis.

Preparation of samples

Each paper currency notes were put, aseptically, in a conical flask containing 10 ml phosphate buffered saline (PBS) for 30 minutes to separate the adhered microbes over the note surface. Then, the notes were taken out aseptically, utilizing sterile forceps and washed. The contents of conical flasks were used to determine bacterial load and detection of bacteria. The currency was reused after drying.

Bacterial culture and enumeration

Total viable count (TVC)

Total viable count (TVC) was performed by inoculation of paper currency samples diluted in PBS. Stock solution of 10^{-1} dilution was prepared by mixing 1 ml of washed sample content in 9 ml of sterile PBS. Ten serial fold dilution (10^{-1} to 10^{-6}) were prepared for each preceding dilution. A 0.1 ml aliquot from each dilution was inoculated onto the center of Petri dishes of nutrient agar (NA) and spread by a glass stick smoothly. Then it was kept in incubator at 37°C for 24 h in an invert position. After 24 h of incubation, colonies on NA were counted and recorded in cfu/paper currency of samples.

Isolation and determination of Staphylococcus* spp. *load

Staphylococcus spp. was enumerated and isolated by incubating the sample in Mannitol-salt agar (MSA). After 24 h of incubation at 37°C, golden yellow colonies were counted and recorded as presumptive *Staphylococcus* spp. in cfu/paper currency. Presumptive *Staphylococcus* spp. colonies on MSA were sub cultured onto freshly prepared MS agar plates and confirmed by Gram's staining where it appeared as cocci shaped grapes like cluster and coagulase test (Murray et al., 2003).

Isolation and determination of Salmonella* spp*. load

Serial dilution of 10^{-1} to 10^{-5} was made in case of *Salmonella* spp. as it can be enumerated in lower dilution than *Staphylococcus* spp. For determination of *Salmonella* load, 0.1 ml aliquot from each dilution was spread on Salmonella-Shigella agar (SSA), followed by 24 hour incubation at 37°C. Colonies that appeared as black center were counted and recorded in cfu/paper currency of samples (FDA, 1992).

Isolation and determination of E. coli load

E. coli detection was carried out using eosin methylene blue (EMB) agar. For enumeration, same serial dilutions prepared for *Salmonella* were spread on EMB agar, followed by 24 h incubation at 37°C. After incubation, metallic sheen colonies were counted on EMB and recorded as presumptive *E. coli.* Metallic sheen colonies were also sub cultured onto freshly prepared EMB agar and confirmed by Gram's staining. On Gram's staining pink colored rod-shaped appearance on microscope proved that was *E. coli* (FDA, 1992).

Antibiotic sensitivity test

Antimicrobial drug susceptibility test against eight commonly used antibiotics in this area included amoxicillin (30 µg), ampicillin (25 µg), azithromycin (30 µg), ciprofloxacin (5 µg), gentamycin (10 µg), norfloxacin (10 µg), tetracycline (30 µg) and erythromycin (5 µg) were performed using disc diffusion method or Kirby-Bauer method (Bauer et al., 1966). The zones of growth inhibition were compared with the zone-size interpretative table standard for *Staphylococcus* spp.*, Salmonella* spp. and *E. coli* provided by Clinical and Laboratory Standards Institute (CLSI, 2016).

Data analysis

All data were managed by incorporating in SPSS software (SPSS-20.0). Descriptive analysis was performed to determine mean and frequency.

RESULTS

Total viable count (TVC)

Table 1 shows the highest total viable count (TVC) of 8.96 log cfu/pc recorded from BDT 10 note collected from egg sellers and lowest value of 6.85 log cfu/pc from BDT 1000 note collected from grocer. According to different occupational groups, the highest (TVC) value of mean log 8.40±0.56 cfu/pc was found in egg seller and lowest value of mean log 7.44±0.32 cfu/pc in grocer. Based on different denomination of paper currency notes, the average (TVC) value ranged from 7.48±0.50 to 8.48±0.60 log cfu/pc; whereas highest one was recorded from BDT 100 notes and lowest one from BDT 1000 notes, respectively.

Staphylococcal count

According to Table 2, the *Staphylococcus* spp. count was highest with 6.89 log cfu/pc in BDT 100 note and lowest value of 5.23 log cfu/pc in BDT 2 note collected from meat seller and egg seller, respectively. Regarding occupational groups, the *Staphylococcus* spp. count was highest with 6.35±0.59 log cfu/pc in fish seller followed by the lowest, 5.40±0.22 log cfu/pc in egg seller. According to different denomination of paper currency notes, the average value of *Staphylococcus* spp. ranged from 5.58±0.42 to 6.10±0.58 log cfu/pc; whereas highest one was recorded from BDT 50 notes and lowest one from BDT 1000 notes, respectively.

Salmonella spp. count

Table 3 presents that the *Salmonella* spp. count of 6.41 log cfu/pc was observed in BDT 10 note collected from egg seller and lowest value of 5.34 log cfu/pc from BDT 20 note collected from meat seller. Among different occupational groups, the highest *Salmonella* spp. count of mean log 5.82±0.37 cfu/pc was estimated in egg seller and lowest value of mean log 5.61±0.06 cfu/pc in fish seller. Regarding different denomination of paper currency notes, the average value of *Salmonella* spp. ranged from 5.36 to 5.88±0.38 log cfu/pc; whereas the highest one was recorded from BDT 10 notes and lowest from BDT 1000 notes, respectively.

E. coli count

According to Table 4, the *E. coli* count was highest of 6.26 log cfu/pc in BDT 50 note and lowest value of 5.18 log cfu/pc in BDT 100 note collected from meat seller and egg seller, respectively. The *E. coli* count was highest with 5.68±0.18 log cfu/pc in fish seller in relation to other occupational groups. According to different denomination

Table 1. Total Viable Count (TVC) on Bangladeshi paper currency notes collected from five occupation groups in Mymensingh city.

Occupation group	Denomination (BDT)								Mean log cfu±SD/paper currency
	2	5	10	20	50	100	500	1000	
Fish seller	7.99	8.81	8.68	7.88	8.25	8.94	7.83	7.10	8.19±0.61
Meat seller	8.20	8.11	7.81	7.65	8.60	8.26	8.88	7.99	8.19±0.40
Egg seller	8.81	8.70	**8.96**	8.65	7.95	8.85	7.68	7.60	**8.40±0.56**
Vegetable seller	8.60	8.20	8.64	8.20	8.58	8.85	7.80	8.35	8.35±0.38
Grocer	6.08	7.70	7.62	7.63	7.75	7.52	7.38	**6.85**	**7.44±0.32**
Average±SD	8.14±0.67	8.31±0.45	8.34±0.59	8.00±0.43	8.23±0.38	**8.48±0.60**	7.91±0.57	**7.48±0.50**	

Table 2. *Staphylococcus* spp. count on Bangladeshi paper currency notes collected from five occupation groups in Mymensingh city.

Occupation group	Denomination (BDT)								Mean log cfu±SD/paper currency
	2	5	10	20	50	100	500	1000	
Fish seller	6.51	6.70	6.64	6.57	6.88	6.67	5.51	5.32	**6.35±0.59**
Meat seller	6.86	6.34	5.48	6.30	6.36	**6.89**	6.18	6.20	6.33±0.44
Egg seller	**5.23**	5.26	5.45	5.36	5.82	5.30	-	-	**5.40±0.22**
Vegetable seller	5.96	5.60	5.30	5.51	6.11	5.70	5.26	5.48	5.61±0.30
Grocer	5.48	5.79	5.65	5.45	5.34	5.26	5.49	5.32	5.47±0.18
Average±SD	6.01±0.68	5.94±0.58	5.70±0.54	5.84±0.56	**6.10±0.58**	5.96±0.77	5.61±0.40	**5.58±0.42**	

Table 3. *Salmonella* spp. count on Bangladeshi paper currency notes collected from five occupation groups in Mymensingh city.

Occupation group	Denomination (BDT)								Mean log cfu±SD/paper currency
	2	5	10	20	50	100	500	1000	
Fish seller	5.58	5.56	5.57	-	5.62	5.70	-	-	**5.61±0.06**
Meat seller	5.95	-	5.51	**5.34**	6.04	5.52	6.26	-	5.77±0.36
Egg seller	6.08	5.60	**6.41**	5.98	5.56	6.11	5.46	5.36	**5.82±0.37**
Vegetable seller	5.79	5.58	6.08	5.83	5.60	5.53	-	-	5.74±0.21
Grocer	5.95	5.60	5.85	5.93	5.28	-	-	-	5.72±0.29
Average±SD	5.87±0.19	5.59±0.02	5.88±0.38	5.77±0.29	5.62±0.28	5.71±0.27	5.86±0.56	5.36	

of paper currency notes, the average value of *E. coli* varied from 5.40±0.20 to 5.84±0.20 log cfu/pc; while the highest one was recorded from BDT 10

Table 4. *E. coli* count on Bangladeshi paper currency notes collected from five occupation groups in Mymensingh city.

Occupation group	Denomination (BDT)								Mean log cfu±SD/paper currency
	2	5	10	20	50	100	500	1000	
Fish seller	5.69	5.76	5.72	5.79	5.75	5.89	5.58	5.28	**5.68±0.18**
Meat seller	5.70	5.72	5.75	5.36	**6.26**	5.46	5.48	5.63	5.67±0.27
Egg seller	5.48	5.68	5.66	5.70	5.95	**5.18**	5.38	5.28	**5.50±0.20**
Vegetable seller	5.67	5.60	5.95	5.11	5.51	5.68	-	-	5.59±0.28
Grocer	5.59	5.66	6.15	5.20	5.48	-	-	-	5.62±0.34
Average±SD	5.63±0.09	5.68±0.06	5.84±0.20	5.43±0.30	5.73±0.31	5.55±0.31	5.48±0.10	5.40±0.20	

Table 5. Prevalence of *Staphylococcus* spp., *Salmonella* spp. and *E. coli* on Bangladeshi paper currency notes in Mymensingh city.

Microorganism	Fish seller (n=8)	Meat seller (n=8)	Egg seller (n=8)	Vegetable seller (n=8)	Grocer (n=8)	Total (n=40)
Staphylococcus spp.	8 (100%)	8 (100%)	6 (75%)	8 (100%)	8 (100%)	**38(95%)**
Salmonella spp.	5 (62.5%)	6 (75%)	8 (100%)	6 (75%)	5(62.5%)	**30 (75%)**
E. coli	8 (100%)	8 (100%)	8 (100%)	6 (75%)	5(62.5%)	**35(87.5%)**
Total	(21)87.5%	(22)91.67%	(22) 91.67%	(20)83.33%	(18)75%	**(103)85.83%**

notes and lowest one from BDT 1000 notes, respectively.

Prevalence of bacteria

Table 5 demonstrates that 85.33% paper currency notes were contaminated with at least one of the organisms tested. Prevalence of *Staphylococcus* spp. was highest (95%) followed by *Salmonella* spp. (75%) and *E. coli* (35%) on paper currency notes.

Antibiotic sensitivity test

Thirty-eight bacterial isolates of three organisms comprising 20 *Staphylococcus* spp., 10 *Salmonella* spp. and 8 *E. coli* isolates were isolated through Gram's staining and biochemical test. All isolates were subjected to antimicrobial susceptibility test against 8 commonly used antibiotics. Table 6 represents the detail results of antibiotic sensitivity test of previously mentioned organisms. *Staphylococcus* spp. was found resistant to ampicillin (100%), amoxicillin (100%), and ciprofloxacin (80%) and sensitive to gentamycin (90%), azithromycin (80%), tetracycline (80%), erythromycin (80%) and norfloxacin (60%). All the isolates of *Salmonella* spp. showed resistant to ampicillin (100%), ciprofloxacin (87.5%), gentamycin (75%), erythromycin; whereas sensitive to azithromycin (87.5%), tetracycline (75%) and norfloxacin (75%). *E. coli* were shown resistant to ampicillin (100%), amoxicillin (100%) and almost resistant to ciprofloxacin (80%), erythromycin (70%) and gentamycin (60%). Most drugs like azithromycin, norfloxacin and tetracycline were found to be sensitive to *E. coli.*

DISCUSSION

Microbial load of total mean bacterial count, *Staphylococcus* spp. *Salmonella* spp. and *E. coli* counts were ranged from 7.48±0.50 to 8.48±0.60 log cfu/pc, 5.58±0.42 to 6.10±0.58 log cfu/pc, 5.36 to 5.88±0.38 log cfu/pc and 5.40±0.20 to 5.84±0.20 log cfu/pc, respectively from all denomination paper currency notes. The highest mean TVC of 8.40±0.56 log cfu/pc counts were obtained from egg seller and the lowest 7.44±0.32 log cfu/pc from grocer. The highest mean

Table 6. Antimicrobial resistance pattern of bacterial isolates from Bangladeshi paper currency notes Mymensingh city.

Organisms	Antibiotics tested							
	No (%) of resistance							
	AMP	GEN	CIP	AZM	TE	NX	E	AMX
Staphylococcus spp.(n=20)	20(100)	2(10)	16(80)	4(20)	6(20)	8(40)	20(20)	20(100)
Salmonella spp.(n=8)	8(100)	6(75)	7(87.5)	1(12.5)	2(25)	2(25)	6(75)	5(62.5)
E. coli(n=10)	10(100)	6(60)	8(80)	2(20)	4(40)	3(30)	7(70)	10(100)

Legends: AMP=Ampicillin; GEN=Gentamycin; CIP=Ciprofloxacin; AZM=Azithromycin; TE=Tetracycline; NX=Norfloxacin; E=Erythromycin and AMX=Amoxicillin

Staphylococcus spp. count of 6.35±0.59 log cfu/pc and lowest value of 5.40±0.22 log cfu/pc were recorded for fish seller and egg seller respectively. The highest mean *Salmonella* spp. count of 5.82±0.37 log cfu/pc was observed in egg seller and lowest of 5.61±0.06 log cfu/pc was observed in fish seller. The highest mean *E. coli* count of 5.68±0.18 log cfu/pc and lowest value 5.50±0.20 log cfu/pc were recorded for fish seller and egg seller, respectively. The previous authors observed more or less similar findings. Feglo and Nkansah (2010) observed the highest mean viable bacterial count of 4.60 log cfu/Note, the GH¢5 4.25 log cfu/Note, and then the GH¢10 had 3.44 log cfu/Note in Ghanaian currency notes. Akond et al. (2015) also found the load of *Salmonella* spp., *Shigella* spp., *Vibrio* spp., *Pseudomonas* spp. and *Staphylococcus* spp. ranging between 0 to 8.39 log cfu/cm^2; 0 to 8.17 log cfu/cm^2; 7.88 to 9.20 log cfu/cm^2; 7.65 to 8.91 log cfu/cm^2 and 7.12 to 7.55 log cfu/cm^2, respectively in Bangladeshi paper currency notes. The presence of *Staphylococcus* spp. on Bangladeshi paper currency notes could be due to rubbing off or surfing from a skin flake. Its natural inhabitant is the human body and is easily transferred to other persons during handling of paper currency notes. As saprophytes, in some cases, they are found elsewhere like air, water, milk and fomites. It can enter into the body through breaks, cuts and abrasions (Umeh et al., 2007). *Salmonella* spp. and *E. coli* are the two enteric pathogens recovered from Bangladeshi paper currency might play a huge role in scattering various infections. These microorganisms can cause cholera, diarrhea, septicemia and urinary tract infection (Ahmed et al., 2010).

The results of the study revealed that 85.83% tested paper currency notes were found to be contaminated by *Staphylococcus* spp. with 95% followed by *E. coli* (87.5%) and *Salmonella* spp. (75%). Firoozeh et al. (2017) reported 77.7% bacterial contamination in 337 Iranian paper currency notes, which is slightly lower than the present study. Akon et al. (2015) observed higher bacterial contamination of 93.70% in 506 Bangladeshi paper currency notes. Yazah et al. (2012) revealed *Staphylococcus aureus* (22.5%), *Escherichia coli* (12.5%) and *Klebsiella* spp. (5%) from 160 different Nigerian paper note that are comparatively lower than the current study. Paper currency notes collected from fish seller, meat seller, egg seller, vegetable seller and grocer were contaminated with *Staphylococcus* spp. at the rate of 100, 100, 75, 100 and 100%, with *Salmonella* spp. at the rate of 62.5, 75, 100, 75 and 62.5%, and with *E. coli* at the rate of 100, 100, 100,75 and 62.5% respectively.

The highest level of (91.67%) contaminants were recovered from meat seller and vegetable seller in comparison to other groups. The findings of the present study supports the findings of Ahmed et al. (2010), who also found that taka collected from fish sellers, meat sellers, vegetable sellers, food vendors and shop keepers were contaminated with *E. coli* at the rate of 69.23, 69.23, 63.63, 50 and 50%; with *Salmonella* spp. at the rate of 42.85, 38.46, 18.18, 0.0 and 0.0% and *Staphylococcus aureus* at the rate of 7.14, 53.84, 9.09, 16.67 and 33.33%, respectively. In Iran, Shekarforoush et al. (2009) also isolated *E. coli, S. aureus and Bacillus cereus* with the prevalence of 13.2, 32.5 and 10.8%, respectively of the 120 Iranian currencies.

The denomination of paper currency notes has a strong correlation with the level of contamination as higher denomination notes had the less contaminant. The outcomes appeared in Table 1-4 indicated that all the paper currency notes had bacterial contamination. BDT 50, BDT 100, BDT 500 and BDT 1000 had lower microbial load in compare to BDT 2, BDT 5, BDT 10 and BDT 20. The vast majority of the general people of Bangladesh among every monetary class frequently utilizes lower paper currency notes in daily activities. Higher paper currency notes are not used as oftentimes as lower category notes. Probably this is the reason while there is higher microbial load in lower paper currency notes. Paper currency notes collected from fish seller, meat seller, egg seller and vegetable seller had the highest percentage of contamination. Since they do not follow the proper hygienic measure during handling of currency, that is the significant concern particularly in regard to wellbeing status of the population. Similar findings were reported by Ahmed et al. (2010) who observed that only BDT 2, BDT 5 and BDT 10 contained high bacterial load. Ali et al. (2015) additionally discovered same results in Pakistani currency notes as the lower denominations had more than higher

denominations.

Nowadays, antimicrobial resistance has become a burning issue throughout the world. Indiscriminate use of antibiotics has leads to treatment failure and augment health cost (Sharma and Dhanashree, 2011). Paper notes are usually contaminated with pathogenic microorganisms in circulation, of which most of them are resistant to commonly used antibiotics reported elsewhere (Firoozeh et al., 2017). Transmission of these antibiotic resistance microorganisms from one individual to another through paper currency may cause serious public health hazards. In the current study, bacteria isolated from Bangladeshi paper currency notes were subjected to antimicrobial susceptibility test against 8 commonly used antibiotic; revealing that most of the antibiotic likes amoxicillin, ampicillin and ciprofloxacin were non-effective against *Staphylococcus* spp. *Salmonella* spp. and *E. coli,* whereas it is sensitive to azithromycin and norfloxacin. This study completely agrees with the previous study conducted by Ali et al. (2015) who observed higher resistance of *Salmonella* spp. against gentamycin and amoxicillin. The resistance of all isolates of the present study to amoxicillin, ampicillin and ciprofloxacin support the studies of Akond et al. (2015) and Oluduro et al. (2014). Therefore Bangladeshi paper currency notes might have a significant relationship in spreading of antibiotic resistant organisms. Multi drug resistant bacteria represent a major threat to human survival and continued existence in connection to bacterial contamination and illness. Thus, increased number of antibiotic resistant bacteria in Bangladeshi currency notes reflects a frightful circumstance for health strategy makers. The outcomes from this investigation demonstrates that Bangladeshi paper currency notes in circulation are contaminated with several bacteria, most of which are resistant to commonly prescribed antibiotics which represent risks to individuals handling paper currency notes.

Conclusion

All the paper currency notes analyzed in this investigation were found to be tainted with at least one or two microscopic microorganisms including *Staphylococcus* spp., *Salmonella* spp. and *E. coli* where most of them are resistant to commonly used antibiotic and in addition pose public health hazard to the individual handling paper money. Thus, care must be taken with caution during the preparation and handling of food to stay away from contamination. Individual cleanliness should be maintained to reduce risk of infection particularly for those who frequently handle food and money. Fish sellers and butchers, as well as other common people should be educated to avoid possible cross contamination between currency notes and food. Regular microbial testing of paper currency notes should be established for large-scale substitution of tainted currency. Children must be kept away from dealing with money notes and adults should abstain from utilizing saliva during counting of currency notes. Introduction of plastic currency notes might be an alternative to paper currency notes, as it can be washed easily.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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