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ALCOHOL DISRUPTED AND INCREASED ANTIBIOTICS RESISTANT PROFILES OF SELECTED BACTERIA RESIDENT IN GASTRO-INTESTINAL TRACT OF WISTER RAT

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ABSTRACT

The study is aimed at investigating the effect of alcohol intake on gut microbiota and antibiotic susceptibility of some of the microbial genera isolated. Twenty-four wistar rats were administered orally with branded dry gin (40% alcohol) and one was picked randomly every 48hrs and sacrificed by cervical decapitation. The intestine was collected aseptically after laparotomy and placed in a sterile petri dish. The intestinal bacteria were enumerated, isolated and identified using standard microbiological methods. The antibiotic susceptibility was done using the disc agar diffusion method. The Total Heterotrophic (x 10^4 CFU/g) and Total Coliform Counts ranged from 2.0 – 65 and 1 – 28 respectively. Bacteria genera isolated are; *Escherichia, Proteus, Citrobacter, Lactobacillus, Enterococcus, Enterobacter, Salmonella, Klebsiella* and *Bacillus. E. coli* was the most (100%) prevalent. *Enterococcus, Lactobacillus* and *Enterobacter* were also highly prevalent. The occurrence of the genera was in the order *Lactobacillus* > *Citrobacter* > *Klebsiella* and *Salmonella* > *Proteus.* All isolates were present in all the intestines few days after administration of the gin, but there was a decline in the occurrence till the 21st day, after which there was an increase. The susceptibility of *Escherichia* to conventional antibiotics was between 0 – 90% and there was no particular trend with the length of exposure but there was substantial decrease in the susceptibility to the antibiotics. The susceptibility of *Enterococcus* to the antibiotics decreased with the length of exposure to the alcohol. Intake of alcohol has a pronounced effect on the gut microbiome and the antibiotic susceptibility of isolates.

Keywords: microbiota, alcohol, antibiotic susceptibility

INTRODUCTION

The Gastrointestinal Tract (GIT) is one of the interfaces existing between a host, environmental factors and antigens in humans. Foods, drinks and microorganisms pass from the environment into the GIT of humans. This imposes threat on the gut (Bengmark, 1998). The gut microbiota is composed of bacteria, eukaryotes and archeae which co-exist with the host to form an intricate and beneficial association (Backhead, 2005).The humans to bacteria cells ratio has been estimated to be 1:1 (Sender *et al.,* 2016).

The roles of the gut microbiome include protection against pathogens, host immunity regulation and gut shaping and strengthening (Gensoleen *et al.,* 2010). Some help in expression of gene, chemotaxis, differentiation, proliferation and apoptosis (Correa-Olivera *et al.,* 2016). They are involved in the fermentation of starch (Lin and Zhang, 2017), vital to the production of vitamins (LeBlanc *et al.*, 2013) and the development of intestinal mucosal and immune system (Hevial *et al*., 2015). The gut microbiota may be affected by lifestyle, diet, host genetics and antibiotics (Ehwarieme *et.al*, 2020; Li *et al.,* 2014). Vassallo (2015) reported that the composition of bacterial species in the gut is influenced by lifestyle, medication, age and diet. The functions of the microbiota can be disrupted by changes in the composition of the microorganisms in the gut (dysbiosis) (Chang and Lin, 2016) and metabolic functions as a result of alcohol consumption.

Alcohol consumption is widespread and found to be a global problem due to abuse by consumers. It is consumed for social reasons, culture and customs and for health reasons. About 2.3billion people consume alcohol daily and over 75million have one form of alcohol disorders (WHO, 2014). Intake of alcohol was reported to have an effect on dysbiosis of the gut microbiome both quantitatively and qualitatively (Yan *et al.,* 2012). Mutlu *et al.* (2012) and Bajaj. (2019) reported that chronic alcohol consumption in humans results in bacterial overgrowth which can lead to dysbiosis. When the gut microbiome is disrupted, there may be an increased susceptibility to pathological changes (Lozupone *et al.,* 2012). Alcohol consumption can result in tissue injury and dysfunction of organs (Purohit, 2008), as well as improper functioning of the immune system thus increasing the risk of infections (Szabo and Mandrekar, 2009). Change in the composition of the microbiome due to intake of alcohol was reported to be responsible for an increase in gut permeability and translocation of the components of the microorganism into circulation (Patrick *et al*., 2018). Dysbiosis induced by alcohol has been reported to be implicated in health complications (David *et al*., 2014).

Other factors apart from toxicity such as changes in the normal composition and functioning of the intestinal microbiota which are induced by alcohol have also been reported to be implicated and responsible for health complications (David *et al.,* 2014). Alcohol was found to be a disruptor of the intestinal microbiota (Bull-Otterson *et al.,* 2013).

There is a crisis of antibiotic resistance even after many decades of discovering the use of antibiotics making the treatment of infections difficult (Lee and Ventolla, 2015). Drug resistance has been reported in microorganisms such as *Enterobacteriaceae, Citrobacter, Enterococcus* and *Streptococcus* (Lee and Ventolla, 2015) and many of them are found in the gastrointestinal tract. Antibiotic resistance is a big threat to food security, food development and global health (Golkar *et al*., 2014). Could this have resulted from dysbiosis as a result of alcohol intake? Steps to manage these threats becoming a crisis are greatly needed.

There is a very limited literature on the effect of alcohol intake on the antibiotic susceptibility/resistance profile of the intestinal microbiota. The study is, therefore, aimed at

investigating the effects of alcohol intake on the gut microbiome and antibiotic susceptibility of some of the genera isolated.

METHODOLOGY

Laboratory Animals Used

A total of twenty four Wistar rats of about 8-10weeks old were purchased from the Animal House Unit of Faculty of Basic Medical Sciences, Delta State University (DELSU), Abraka. The rats were housed in well aerated plastic cages in the Department of Microbiology, DELSU, Abraka, Nigeria at (28 ± 2ºC, relative humidity 60-70 %, 12hr light/ dark cycle). During the whole period of study, the animals were supplied with standard grower mash diet (Composition of the grower's marsh: Protein -19.0% Fat -2.85% Fibre -6.00% Calcium - 1.00% Available phosphate -0.45% Energy -2875 KGC (Animal Care Services Konsult [NIG LTD], Asaba, Delta State) and water *ad libitum*, in standard wire meshed wooden cages for 10 to 12 days prior to commencement of the experiment. In this study, all animal experiments were conducted in compliance with the National Institute of Health Guidelines for Care and Use of Laboratory Animals (Publication No. 85 - 23, revised 1985) and approved.

Administration of Dry Gin and Sample Collection

Branded dry gin (40% alcohol) was administered orally to the rats and one was picked randomly every 48hrs, after an overnight fast. The rats were sacrificed by cervical decapitation and each rat was placed on its dorsal surface, and a laparotomy was carried out to expose the internal organs, and intestine was collected aseptically into a petri dish. The control was sacrificed before the administration of the dry gin.

Isolation of Bacteria

An albino mouse was randomly selected, weighed, sacrificed and aseptically dissected. The gastrointestinal tract was collected and homogenized using pestle and mortar. One gram of the homogenate was serially diluted and 10^{-4} dilution was introduced into nutrient agar and MacConkey agar plates. The plates were incubated at 37^0C for 24hrs. After incubation, distinct colonies were picked and sub-cultured into fresh agar plates to obtain pure culture of the isolates. The colonies were counted and expressed as colony forming units/gram (CFU/g).

Identification of Bacterial Isolates

The isolates were identified on the basis of cultural, morphological and biochemical characteristics. The biochemical tests included Gram staining, motility, catalase, oxidase, indole, citrate utilization, Triple Sugar Iron test and H_2S production.

Antibiotics Susceptibility

This was carried out using the disc agar diffusion method as described by Bauer *et al.* (1966). The isolates that were most prevalent (*Enterococcus* and *Escherichia*) were used for this test. Suspension of each isolate was made in 0.85% NaCl and adjusted to the turbidity of Mcfarland standard solution. The standardised isolate was used to inoculate the surface of Mueller-Hinton agar plates using sterile cotton swab stick. The plates were allowed to stand for about 30mins after which antibiotics sensitivity discs containing Ampicillin, Ceporex, Tarivid, Nalidix, Perfloxacin, Gentamycin, Augmentin, Ciprofloxacin, Septrin and Streptomycin (Abtek) were aseptically placed on the plate with the aid of sterile forceps. The plates were inverted and incubated at 37^0C (Slevamohan and Sandhya, 2012).

RESULTS AND DISCUSSION

The mean value of the Total Heterotrophic Counts (THC) was between 2.0 x10^4 CFU/ml and 65x10^4CFU/ml (Table 1). The highest value was recorded in the control when the alcohol had not been administered. There was subsequent reduction in the population. By about the 8th - 15th day, there was an increase followed by significant decrease by the 19th – 25th day and again there was a peak by the 27th day. The Total Coliform Counts (TCC) were lower than the THC. There was a decrease in the TCC between day 0-2, after which the values were increasing till the 19th day. By the 19th day, there was significant reduction, followed by a spike by the 25th day (Table 1).

The bacteria isolated were *Escherichia coli*, *Proteus* sp., *Citrobacter* sp., *Enterococcus* sp., *Enterobacter* sp., *Klebsiella* sp., *Salmonella* sp. and *Lactobacillus* sp. There was more than 70% occurrence of the isolates from the 2nd day to the 6th day. By the 8th day, many of the isolates were not present. By the 25th day, all the isolates had 100% occurrence. From the 8th day to the 21st day, there was significant reduction (from 100% - 33%) in the isolates that were in the GIT (Table 2). After the 6th day, the microbial diversity reduced significantly till about the 21st day, after which there was an increase and subsequently all the isolates were represented.

The initial decrease in the total heterotrophic count (microbial population) may be attributed to the alcohol having an inhibitory effect on the growth of the organism but over time, some of the organisms would have adapted to the alcohol. Also, a decrease in the concentration of alcohol would have occurred hence, enhancing growth and subsequent increase in the total heterotrophic count. This is similar to the report of Patterson and Rick. (2014) that ethanol had a significant inhibitory effect on some bacteria species. Ribeiro *et al*. (2015) reported that alcohol at 60 - 80% can kill a broad range of germs including bacteria, fungi and viruses. The inhibitory effect of alcohol on microbial growth may be explained by the fact that alcohol is able to kill bacteria and other organisms through denaturation of proteins and interference with the enzymes. Shasmal *et al.* (2016) reported that protein denaturation destroys dehydrogenase of *E. coli* and increases the lag phase of *Enterobacter aerogenes*. *E. coli, Serratia sp.* were killed by all concentrations of ethanol from 40-100% while *S. aureus* and *Streptococcus* were killed in 10s by 60-90% alcohol.

The reason for the non-inhibition of some of the isolates may be due to the ability of the organisms to exhibit resistance to the alcohol. This is similar to the work of Ingram. (1990) that some microorganisms were able to survive in the presence of alcohol which was attributed to adaptation and evolutionary changes in the cell membrane composition and that different cellular activities vary in their tolerance to ethanol. The organisms isolated were found to be more of rods especially gram negative rods and were found to reduce in number over time. This can be attributed to alcohol dissolving the lipids found in their outer cell membrane beneath the thin peptidoglycan cell wall.

The alcohol had a significant effect on the antibiotic susceptibility of *Enterococcus* sp. and *E. coli* as shown in Tables 3 and 4 respectively. *Escherichia coli* was found to be susceptible to all the conventional antibiotics before the administration of the alcohol. There was a high incidence of resistance to the antibiotics after the administration of the alcohol. There was a similar trend for *Enterococcus.*

Escherichia exhibited resistance to more than one hence exhibiting multidrug resistance.

CONCLUSION

The intake of alcohol had a pronounced effect on the microbial population and the diversity of genera of the microorganisms. There was an initial decrease in the Total Heterotrophic Counts and the Coliform Count. There was a significant effect on antibiotic susceptibility of the most prevalent genera (*Escherichia* and *Enterococcus*) which increased with time. The organisms exhibited multi-drug resistance. Alcohol disrupted the microbiota and diversity of organisms found in the mice GIT and had a significant effect on the antibiotic profiles of *Enterobacter* and *Escherichia*.

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TABLES

Table 1: Total Heterotrophic and Coliform Counts (x10^4 CFU/g)

Samples	Day	THC	TCC
M_1(control)	0	65	7
M_2	2	5	4
M_3	4	4.5	2
M_4	6	2.0	1
M_5	8	26	1
M_6	10	28	6
M_7	12	32	10
M_8	15	34	17
M_9	17	36	18
M_{10}	19	25	21
M_{11}	21	26	26
M_{12}	23	43	28
M_{13}	25	49	25
M_{14}	27	54	22

Key: THC=Total Heterotrophic Count; TCC=Total Coliform Count

Table 2: Occurrence of Isolates from rat GIT

Days	*E. coli*	*Proteus* sp.	*Citrobacter* sp.	*Lactobacillus* sp.	*Enterobacter* sp.	Bacillus sp.	*Klebsiella* sp.	*Salmonella* sp.	*Enterococcus* sp.	Percentages
control-day 0	+	+	+	+	+	+	+	+	+	100
2	+	+	+	+	+	+	+	+	+	100
4	+	+	+	+	-	-	+	+	+	77.78
6	+	+	+	+	-	+	+	+	+	88.89
8	+	-	-	+	+	-	-	-	+	44.44
10	+	-	-	+	-	-	-	-	+	33.33
12	+	-	-	+	+	-	-	-	+	44.44
15	+	-	-	-	-	+	-	-	+	33.33
17	+	-	+	-	+	-	-	-	-	33.33
21	+	-	+	-	+	-	-	-	-	33.33
23	+	-	-	+	+	-	+	+	+	66.66
25	+	+	+	+	+	+	+	+	+	100
27	+	+	+	+	+	+	+	+	+	100
% occurrence of each isolate	100	42.8	57.1	85.7	64.3	42.8	50.0	50.0	92.9	

Key: +=present; -=absent

Table 3: Antibiotic Susceptibility of *Escherichia* sp. isolated from rat GIT

	Zones of Inhibition(mm)											
Days	PN	CEP	OFX	NA	PEF	CN	AU	CPX	SXT	S	**S(%)**	**R(%)**
0	23	21	21	20	22	22	23	22	22	17	100	0
2	18	15	20	3	0	15	0	0	10	0	30	70
4	17	20	20	5	15	20	0	0	5	10	50	50
6	15	15	15	0	15	15	0	0	10	10	50	50
8	8	10	0	4	0	0	0	0	0	0	0	100
10	10	14	15	0	0	4	0	0	0	0	20	80
12	0	8	12	0	10	13	0	0	0	0	10	90
15	20	20	20	5	7	12	3	0	20	0	40	60
17	15	12	20	0	10	18	0	5	0	0	30	70
19	0	5	14	10	5	7	0	0	0	0	10	90
21	20	0	15	15	0	8	4	0	4	0	30	70
23	11	6	13	0	10	14	0	10	0	0	20	80
25	20	18	20	0	10	20	5	2	20	3	50	50
27	12	15	12	4	0	7	0	0	12	0	10	90

Key: S = Sensitivity; R = Resistance; PN = Ampicillin; CEP = Ceporex; OFX = Tarivid; NA = Nalidix; PEF = Perfloxacin; CN = Gentamycin; AU = Augmentin; CPX = Ciprofloxacin; SXT = Septrin; S = Streptomycin.

Table 4: Antibiotic Susceptibility of *Enterococcus* sp. isolated from rat GIT

	Zones of Inhibition (mm)											
Days	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E	**S(%)**	**R(%)**
0	20	17	18	17	15	5	20	20	20	20	90	10
2	10	5	15	4	12	0	10	20	20	20	40	60
4	10	14	10	8	15	7	10	20	20	20	50	50
6	10	0	13	0	15	7	15	20	20	20	60	40
8	5	5	7	12	0	0	20	18	20	20	40	60
10	0	0	0	10	4	0	10	15	18	20	30	70
12	4	0	0	0	0	0	3	10	15	20	30	70
15	3	0	0	3	0	0	5	18	20	20	30	70
17	0	0	3	3	0	0	0	15	20	18	30	70
19	0	0	7	0	0	0	5	15	20	20	30	70
21	0	0	0	0	0	0	0	15	18	18	30	70
23	0	0	0	0	0	0	0	10	18	18	20	80
25	0	0	15	0	0	0	5	10	20	20	20	80
27	5	0	17	0	5	0	7	10	20	20	20	80

Key: S = Sensitivity; R = Resistance; PEF = Perfloxacin, CN = Gentamycin; APX = Ampiclox; Z = Zinnacef; AM = Amoxicillin; R = Rocephin; CPX = Ciprofloxacin; S = Streptomycin; SXT = Septrin; E = Erythromycin