

PARASITOLOGICAL, BACTERIAL AND FUNGAL EVALUATION OF SOME READY-TO-EAT FOODS SOLD BY VENDORS IN PORT HARCOURT, RIVERS STATE.

ABSTRACT

Aims: To determine the prevalence and distribution of gastrointestinal parasites, bacteria and fungi in Date, Wet Tiger nut, Dry Tiger nut, Kuli-kuli and kola nuts sold by roadside vendors in nine locations in Rivers State.

Study Design: A total of 270 samples were procured randomly from roadside sellers.

Place and Duration of Study: Department of Animal and Environmental Biology [parasitology unit], University of Port Harcourt, between March and July 2022.

Methodology: The 270 samples were examined for gastrointestinal parasites using direct wet preparation and sedimentation techniques, while 135 of the samples selected based on type and location were examined for bacteria and fungi using standard microbial techniques.

Results: Out of the 270 samples examined, 124 (49.9%) were positive for gastrointestinal parasites as follows; *Ascaris lumbricoides* 52 (19.3%), *Ancylostoma* spp 39 (14.4%), *Trichuris trichiura* 19 (7.0%) and *Giardia lamblia* 14 (5.2%). Dry Tiger nut recorded the highest prevalence 39 (72.2%) and Kola nut 11 (20.4%) had the least prevalence. Aluu had the highest prevalence 21 (70.0%) while Rumuokoro recorded the least 3 (10.0%) $p=0.05$. Of the 135 samples, 96 (71.1%) was positive for bacteria while 19 (14.1%) was positive for *Candida* species (fungi). The bacteria isolated were *Escherichia coli* 48 (35.5%), *Klebsiella* species 32 (23.7%) and *Staphylococcus aureus* 16 (11.9%). Kola nut recorded the highest bacterial contamination 26 (96.3%) and Date the lowest 10 (37.0). Samples from Choba had the highest bacterial contamination 12 (80%) while Alakahia had the least bacterial contamination 7 (46.7%) $p=0.05$. Dry Tiger nut 12 (44.4%) and Wet Tiger nut 7 (25.9%) were the only sample types with fungal contamination.

Conclusion: Vendors and consumers should be educated on the possible health implications of poorly handled RTE foods, and government should implement and enforce sanitary rules to preserve the environmental quality of areas where RTE foods are being processed and sold.

Keywords: Bacterial, Parasitological, Fungal, Read-to-Eat foods, Port Harcourt.

1. INTRODUCTION

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Ready-to-eat foods are foods that are consumed at the point of sale or later, without any further processing or treatment (1). They include some vegetables, fruits, tubers, nuts, etc. They are a special dietary source of nutrients, vitamins and fiber for humans and are thus vital for health and wellbeing. Ready-to-eat foods like fruits and nuts are an important portion of the human diet and are often being contaminated with both pathogenic and non-pathogenic microorganisms. (2). In Nigeria, most ready-to-eat food vendors prepare/process foods in unpolished, unhygienic environments with little or no acquaintance about the cause of food borne disease. The washing of ready-to-eat foods is generally carried out in large bowls or buckets, with rodents and insects on-site(3).

Foodborne disease is the result of ingestion of foodstuffs contaminated with microorganisms, parasites, or chemicals, and encompasses a wide spectrum of illness and public health problem worldwide (4). Foodborne illnesses are usually infectious or toxic in nature and are caused when bacteria like *Salmonella*, *Campylobacter* and *Escherichia coli*, parasites such as *Ascaris*, *Cryptosporidium*, *Entamoeba histolytica* or *Giardia*, enter the food chain through water or soil and can contaminate fresh produce. Many foodborne diseases may lead to long-lasting disability and death (5). The 2019 World Bank report on the economic burden of the foodborne diseases indicated that the total productivity loss associated with foodborne disease in low- and middle-income countries was estimated at US\$ 95.2 billion per year, and the annual cost of treating foodborne illnesses is estimated at US\$ 15 billion (5).

The prevalence of foodborne diseases in Nigeria is alarming despite efforts by Government and Non-Governmental Organizations to prevent the spread of foodborne pathogens. Health and socioeconomic implications of foodborne diseases are enormous, including loss of productivity and low quality of life (6). Foodborne diseases presented the first-ever estimates of disease

burden caused by 31 foodborne agents (bacteria, viruses, parasites, toxins, and chemicals) at global and sub-regional level, highlighting that more than 600 million cases of foodborne illnesses and 420 000 deaths could occur in a year. The burden of foodborne diseases falls disproportionately on groups in vulnerable situations and especially on children under 5, with the highest burden in low- and middle-income countries (5). In a 2019 study in Gondar town, Ethiopia to evaluate the bacteriological profile, antimicrobial susceptibility patterns of the isolates among street vended foods, 72 food samples of street vended ready-to-eat food were analyzed for bacteria pathogens and 44/72 tested positive for the presence of bacteria, the pathogens found were *E. coli* (23.8%), *S. aureus* (53.96%), *Enterobacter species* (15.87%), and *Citrobacter species* (6.3%), the presence of these bacteria in foods could lead to potential health problems for consumers(7).

Ready-to-eat foods are widely consumed as choice meals especially by school aged children and the fast-paced working class in most low- and middle-income countries (LMICs), where they contribute substantially to the dietary intake. Depending on the type of processing and packaging material, ready-to-eat food could be industrially or traditionally processed. Typically, ready-to-eat food vendors are of low literacy level, as such, they lack knowledge about good hygiene and food handling practices. In addition, ready-to-eat foods are often vended in outdoor environments such that they are exposed to several contaminants of microbial origin (8). A study was carried out in Nasarawa state, Nigeria to check for the fungal species associated with Date palm fruit and Tiger nut, and the fungal genera found were *Aspergillus*, *Penicillium*, *Rhizopus*, and *Mucor*. This indicates that Tiger nuts and Date fruits sold in Lafia market presented heavy burden of fungal contaminations and these contaminants have the capacity of releasing harmful metabolites into the fruits thus making them unsafe for consumption (9). Depending on the

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quantity and type of food contaminant, consumption of contaminated ready-to-eat foods may result in foodborne diseases and several other adverse health effects in humans. This could constitute major hurdles to growth and development in Low- and middle-income countries (8). Despite the reported potential health risk associated with ready to eat food contamination by pathogens, there has been limited research on the evaluation of the parasitological, bacteriological and fungal contamination all done on a particular set of selected ready to eat foods sold by vendors. Hence, this study evaluates the distribution of parasites, bacteria and fungi in *Phoenix dactylifera* (Date), *Cyperus esculentus* (Wet tiger nut), *Cyperus esculentus* (Dry tiger nut), *kuli-kuli* (Peanut cake) and *Cola nitida* (Kola nut) sold by vendors in Port Harcourt, Rivers State.

2. MATERIAL AND METHODS

2.1 STUDY AREA

This study was conducted in Port Harcourt metropolis, Rivers state, Nigeria. Located at 4.824167°N, 7.033611°E of the Greenwich. Port Harcourt metropolis is situated in the southern part of the country, in the delta of the Niger River. The inland part of the state consists of tropical rainforest and towards the coast is the typical Niger Delta environment having many mangrove swamps while the riverine part of the state has three hydro-vegetation zones such as beach ridge, salt water and fresh water. The major occupations of the indigenous people are agriculture (fishing, animal husbandry, farming, palm oil processing), petty trading, private businesses like owning provision stores, hotels, bars, restaurants, roadside vending of ready-to-eat food (may be hawked around in wheelbarrows, or stationary). A respectable number of the indigenes are public servants.

2.2 SAMPLE COLLECTION

A total of 270 samples of ready-to-eat foods were randomly collected from vendors and examined for gastrointestinal parasites, 54 samples each of Date, Wet Tiger nut, Dry Tiger nut, *kuli-kuli* and Kola nut from nine different locations; Choba, Alakahia, Aluu, Rumuokwuta, Cattle market Oyigbo, Trailer Park Onne, Rumuola, Rumuokoro and mile 3 market respectively. One hundred and thirty-five of the samples selected based on type and location were examined for bacteria and fungi using standard microbial techniques

2.2.1 SAMPLE PROCESSING

The nuts were soaked in 150 ml of sterile normal saline, in sterile bottles for one hour. After the hour, the nuts were washed by shaking the bottles vigorously. The saline was then transferred into a sterile beaker. The process was repeated for three additional washes and all the washes pooled.

2.2.2 EXAMINATION OF SAMPLES FOR PARASITES

Aliquots of the pooled saline washes were examined microscopically for the presence of parasites as follows;

2.2.3 DIRECT WET PREPARATION METHOD

Using a plastic pipette, normal saline was placed at one end of a clean grease-free glass slide and iodine was placed on the other end and a drop of the sample Aliquot was emulsified at both ends and were covered with cover slips carefully to avoid air bubbles. The smear was ~~viewed~~ examined initially by microscopically using a low power (*10x) objective and then again by

using a high power(~~40x~~) objective lenses respectively. Parasites were identified by the morphological structures of their cysts, ova or larvae using Atlas of Parasitology (10).

2.2.3 SEDIMENTATION TECHNIQUE

Five (5ml) of 10% formalin was transferred into a centrifuge tube followed by 2ml of the sample Aliquot and 3ml of ether was added raising it to the 10ml mark. A stopper was placed at the top of each tube and shaken. The tubes were then centrifuged for 5 minutes at a speed of 2500rpm. After centrifugation, the supernatant was discarded by gently inverting the tubes leaving the deposits in the tube and sediments were then re-suspended. A drop of the deposits was placed on a clean slide and covered with a cover slip for examination under a microscope at X10 and X40 objective lenses respectively. Parasites were identified by the morphological structures of their cysts, ova or larvae using Atlas of Parasitology (10).

2.3 EXAMINATION OF SAMPLES FOR BACTERIA

2.3.1 PREPARATION OF CULTURE MEDIA

The media used were prepared according to the manufacturer's instructions. They were Nutrient Agar (NA), *Salmonella Shigella* Agar (SSA), Sabouraud Dextrose Agar (SDA) and MacConkey Agar (MA).

2.4 CHARACTERIZATION OF ISOLATES

2.4.1 GRAM STAINING OF THE ISOLATES

A smear was prepared from a 24-hour fresh culture and heat-fixed by passing over a flame and subsequently, the smear was flooded with crystal violet for 1 minute and rinsed under slow flowing tap, again, the smear was flooded with gram's iodine for 1 minute and rinsed, it was flooded with ethanol for 20 seconds and rinsed, then finally safranin was added for about 1

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minute and washed off with water. The slides were allowed to air dry and viewed under the light microscope using oil immersion objective lens, cells that appeared purple were recorded as gram-positive cells while cells that appeared pink under the microscope were recorded as gram-negative cells. (11).

2.5 BIOCHEMICAL TESTS FOR IDENTIFICATION OF BACTERIA

The biochemical test involved hydrogen peroxide test, oxidase test, citrate slant and urase test.

2.5.1 OXIDASE TEST

A piece of filter paper was soaked in 1% solution of oxidase reagent (tetramethyl-p-phenylenediamine- dihydrochloride) prepared by standard procedure. Sample of growth from the nutrient agar slant was obtained using sterilized platinum wire loop and smeared on the moistened piece of paper. Development of an intense purple color by the cells within 30 seconds indicates a positive oxidase test.

2.5.2 HYDROGEN PEROXIDE TEST

The test demonstrates the presence of catalase which is an enzyme that catalyzes the release of oxygen from hydrogen peroxide (H_2O_2). A colony of 24 hours old culture was picked using a sterile loop and then emulsified in a few drops of hydrogen peroxide on a clean slide. Presence of effervescence indicated catalase positive reaction whereas negative reaction showed no effervescence.

2.5.3 CITRATE SLANT UTILIZATION TEST

The Citrate test uses a medium in which sodium citrate is the only source of carbon and energy. If an organism can use citrate as the sole source of carbon and energy, it will need to use ammonium salts for nitrogen. This will result in the release of ammonia, causing a color change in the medium from green to blue. Tubes of Simon's citrate agar was each inoculated

with a test organism and incubated at 35^oC for 48 hours. A change in the medium from green to royal blue was recorded as a positive test. (12).

2.5.4 UREASE TEST

Isolates were inoculated into liquid urea agar supplemented with urea and aseptically dispensed into sterile bijoux bottles and slanted to solidify. They were incubated at 37^oC for 24-48 hours. Development of bright pink or red color indicates positive urea reaction.

2.5.5 INDOLE TEST

This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan to indole, which accumulates in the medium. Isolated colony of the test organism was emulsified in tryptophan broth (peptone water) and incubated at 37^oC for 24 hours in ambient air. 0.5 ml of Kovac's reagent was added to the broth culture down the side of the tube and observed for color change at meniscus. The development of a red color (benzaldehyde reagents) within 20 seconds indicates the presence of indole. A negative test is colorless or slightly yellow.

2.6 FUNGAL IDENTIFICATION

The fungal isolates were identified by their distinct appearance using a sabouraud dextrose agar. They were also identified microscopically by placing a drop of the sample Aliquot on a clean grease free slide and covered with a cover slip and viewed at X40 magnification.

2.7 STATISTICAL ANALYSIS

Data collected were analyzed using Microsoft excel 2016 version. The ANOVA analysis was used to determine association between contamination (of parasite and bacteria) and type of samples, and the association between contamination (of parasite and bacteria) and location, while the T-test analysis was used to determine association between fungal contamination and type of

sample, and association between fungal contamination and location. P values less than or equal to 0.05 were considered significant.

3.0 RESULTS&DISCUSSION

3.1 RESULTS

3.1.1 PARASITOLOGICAL ANALYSIS

A total of 270 samples of ready to eat foods; Date, Wet Tiger nut, Dry Tiger nut, Kuli-kuli and kola examined for parasites, 124(45.9%) samples were positive for gastrointestinal parasites as follows; *Ascaris lumbricoides* 52(19.3%), Hookworms 39(14.4%), *Giardia lamblia* 14(5.2%) and *Trichuris trichiura* 19(7.0%) respectively. There was no polyparasitism observed. The ready to eat foods were positive for gastrointestinal parasites as follows;

Dry Tiger nut 39(72.2%), Wet Tiger nut 30(55.6%), Date 27(50.0%), kuli-kuli 17(31.5%) and Kola nut 11(20.4%) respectively. Prevalence of parasites based on location were as follows; Aluu 21(70.0%), Onne 20(66.7%), Oyigbo 19(63.3%), Alakahia 17(56.7%), Rumuokwuta 16(53.3%), Choba 15(50.0%), Rumuola 8(26.7%), Mile 3(16.7%) and Rumuokoro 3(10.0%) respectively, see Table 1.

3.1.2 BACTERIAL ANALYSIS

Of the 135 samples subjected to bacterial analysis, 96(71.1%) were positive for bacteria as follows; *Escherichia coli* 48(35.6%), *Klebsiella species* 32(23.7%) and *Staphylococcus aureus* 16(11.9%) respectively. The ready to eat foods were positive for bacteria as follows; Kola nut 26 (96.3%), Wet tiger nut 21(77.8%), Dry Tiger nut 21(77.8%), kuli-kuli 18(66.7%) and Date 10(37.0%) respectively. Prevalence of bacteria based on location were as follows; Rumuokwuta 13(86.7%), Choba 12(80.0%), Onne 12(80.0%), Rumuola 11(73.3%), Mile 3

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11(73.3%), Oyigbo 10(66.7%), Rumuokoro 10(66.7%) and Alakahia7(46.7) respectively see Table 2.

3.1.3 FUNGAL ANALYSIS

A total of 135 samples were examined for fungi, 19(14.1%) were positive for *Candida* species. The ready to eat foods were positive for bacteria as follows; Dry Tiger nut 12(44.4%) and Wet Tiger7(25.9%), Kola nut, *kuli-kuli* and Date were free from fungal species.

Prevalence of fungi based on location were as follows; Rumuokwuta 7(46.7%), Rumuola 5(33.3%), Choba 4(26.6%), Aluu 2(13.3%) and Rumuokoro1(6.7%) respectively (See Table 3).

3.2 DISCUSSION

This study revealed (49.9%) prevalence gastrointestinal parasites contamination in the ready to eat food samples, this is slightly higher than the findings of (13) who recorded (39.1%) parasitic contamination of fruits and vegetables collected from local markets.

Ascaris lumbricoides recorded the highest prevalence (19.3%) while *Giardia lamblia* had the lowest prevalence, this is in line with the study of (14) who examined the Parasitic contamination of some fruits, vegetables, and nuts sold in Jos, and observed *Ascaris lumbricoides* and *Giardia lamblia* have the highest and least prevalence of the parasites detected.

This study showed the presence of *Escherichia coli* (35.6%), *Klebsiella* species (23.7%) and *Staphylococcus aureus* (11.9%) in ready to eat food, with Wet and dry Tiger nut having both or one of *Staphylococcus aureus* and *Escherichia coli*. This is in agreement with (15) where *Staphylococcus aureus* (25.0%), and *Escherichia coli* (22.7%), were also found in Tiger nut.

Staphylococcus Aureus was found to be the least frequent bacterial isolate (11.9%). Also (16) In Gondar town Ethiopia, noted *Staphylococcus aureus* as the most frequent (53.96%) among the isolates from street vended foods. *Escherichia coli* (13.3%) and *Klebsiella* species (53.3%) were found in some ready-to-eat food samples from mile 3 Port Harcourt. This corroborates the report

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by (17) who also isolated *Escherichia coli* (70.0%) and *Klebsiella species* (50.0%) when they evaluated the microbial composition of some ready to eat food in Mile 3 market, Port Harcourt.

Tiger nut (wet and dry) was positive for *Candida species* at (14.1) which is higher than (9) which recorded a prevalence of (6.61%), and (18) on some microorganisms associated with exposed Tiger nut milk, and recorded a prevalence of (4.35%). Based on location Aluu had the highest prevalence (70.0%) while Rumuokoro recorded the least (10.0%) the other locations recorded between 16-66% parasitic contamination. Samples from Choba had the highest bacterial contamination 12(80%) while Alakahia had the least bacterial contamination (46.7%) p=05. Variation in contamination might be due to the hygiene of the vendors, or the sanitary conditions of the locations where the ready-to- eat foods are being sold which was evident in the study.

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UNDER PEER REVIEW

3.3 Tables

Table 1 Parasitological analysis

Parasite	No. Examined	No. Infected (%)	Hookworm	<i>G. lamblia</i>	<i>A.lumbricoides</i>	<i>T.trichiura</i>	Formatted: Font: Not Italic, Font color: Auto
FOOD TYPE							
Date	54	27 (50.0)	6 (11.1)	0	8 (14.8)	13(24.1)	Formatted: Font: Not Italic
Wet Tiger nut	54	30 (55.6)	9 (16.7)	3 (5.6)	17(31.5)	1 (1.9)	
Dry Tiger nut	54	39 (72.2)	20 (37.0)	0	14(25.9)	5 (9.3)	
<i>Kuli-kuli</i>	54	17 (31.5)	4 (7.4)	9 (16.7)	4 (7.4)	0	
Kola nut	54	11 (20.4)	0	2 (3.7)	9 (16.7)	0	
Total	270	124(49.9)	39(14.4)	14(5.2)	52(19.3)	19(7.0)	
	S.D=12.3	df=4	F=0.7940	F_{crit}=3.49	P=.05		
LOCATION							
Choba	30	15 (50.0)	9 (30.0)	2 (6.7)	4 (13.3)	0	
Alakahia	30	17 (56.7)	5 (16.7)	0	12(40.0)	0	
Aluu	30	21 (70.0)	6(20.0)	2(6.7)	7(23.3)	6(20.0)	
Rumuokwuta	30	16 (53.3)	0	0	8 (26.7)	8 (26.7)	
Oyigbo	30	19 (63.3)	9 (30.0)	7(23.3)	3 (10)	0	
Onne	30	20 (66.7)	5 (16.7)	3(10.0)	10(33.3)	2 (6.7)	
Rumuola	30	8 (26.7)	2 (6.7)	0	5 (16.7)	1 (3.3)	
Rumuokoro	30	3 (10.0)	0	0	1 (3.3)	2 (6.7)	
Mile 3	30	5 (16.7)	3 (10.0)	0	2 (6.7)	0	
Total	270	124(49.9)	39(14.4)	14(5.2)	52(19.3)	19 (7.0)	
	S.D=6.72	df=8	F=0.9070	F_{crit}=2.51	P=05		

Table 2. Bacterial analysis

Bacteria	No. Examined	No. Infected (%)	<i>E. coli</i>	<i>Klebsiella spp</i>	<i>S.aureus</i>
FOOD TYPE					
Date	27	10 (37.0)	10 (37.0)	0	0
Wet Tiger nut	27	21 (77.8)	7 (25.9)	9 (33.3)	5 (18.5)
Dry Tiger nut	27	21 (77.8)	13 (48.1)	8 (29.6)	0
<i>Kuli-kuli</i>	27	18 (66.7)	18 (66.7)	0	0
Kola nut	27	26 (96.3)	0	15 (55.6)	11 (40.7)
Total	135	96 (71.1)	48 (35.6)	32 (23.7)	16 (11.9)
	S. D=35.88	df=4	F=3.7018	F_{crit}=6.39	P=0.05
LOCATION					
Choba	15	12 (80.0)	10 (66.7)	2 (13.3)	0
Alakahia	15	7 (46.7)	0	6 (40.0)	1 (6.7)
Aluu	15	10 (66.7)	7 (46.7)	3 (20.0)	0
Rumuokwuta	15	13 (86.7)	11 (73.3)	0	2 (13.3)
Oyigbo	15	10 (66.7)	2 (13.3)	8 (53.3)	0
Onne	15	12 (80.0)	9 (60.0)	3 (20.0)	0
Rumuola	15	11 (73.3)	3 (20.0)	2 (13.3)	6 (40.0)
Rumuokoro	15	10 (66.7)	4 (26.7)	0	6 (40.0)
Mile 3	15	11 (73.3)	2 (13.3)	8 (53.3)	1 (6.7)
Total	135	96 (71.1)	48 (35.6)	32 (23.7)	16 (11.9)
	S. D=1.73	df=8	F=0.1868	F_{crit}=2.95	P=0.05

Table 3. Fungal analysis

Fungi	No. Examined	No. Infected (%)	<i>Candida spp</i>
FOOD TYPE			
Date	27	0	0
Wet Tiger nut	27	7(25.9)	7(25.9)
Dry Tiger nut	27	12(44.4)	12(44.4)
<i>Kuli-kuli</i>	27	0	0
Kola nut	27	0	0
Total	135	19(14.1)	19(14.1)
S. D= 5.495	df=4	t.stat=1.139	P=05
LOCATION			
Choba	15	4 (26.7)	4 (26.7)
Alakahia	15	0	0
Aluu	15	2(13.3)	2 (13.3)
Rumuokwuta	15	7(46.7)	7 (46.7)
Oyigbo	15	0	0
Onne	15	0	0
Rumuola	15	5(33.3)	5 (33.3)
Rumuokoro	15	1(6.7)	1(6.7)
Mile 3	15	0	0
Total	135	19 (14.1)	19 (14.1)
S. D= 17.46	df=8	t.stat=0.19089	P=0.05

4. CONCLUSION

This study shows that ready to eat food are avenues by which pathogens infect humans thereby posing serious health risks if hygienic handling and level of consumptions aren't checked or if sanitary measures are not properly taken before consumption. The most effective way to curb the level of pathogens found in ready-to-eat food is by proper handling which in turn will prevent the contamination of the food material.

5. RECOMMENDATIONS

- i. The government should make provisions to educate these vendors on health risks of poorly handled food, as well as enforce laws on healthy monitoring of ready-to-eat foods sold by vendors.
- ii. The general public should be educated through news articles, radios, and televisions on the possible dangers of consuming poorly handled ready-to-eat foods, they should be advised to wash properly before consumption so as to reduce or get rid of possible pathogens on the surface of these food materials.
- iii. Further studies should be conducted to check the viability of the pathogens found in ready-to-eat foods and how they are associated directly with diseases.
- iv. Government should also provide portable and free water for the general population so vendors can access for proper washing of these food materials, and also implement the policy of already existing environmental laws on hygiene which will help to curb the breeding of some of these food borne pathogens.

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