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Bacteriological Aspect of Frozen Beef Burger

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Abstract

The present study was carried out on 105 random samples of frozen beef burgers, collected from different localities (Benha city, centers and villages with 35 for each locality) at Kaliobia Governorate, to evaluate the bacterial quality and the hygienic health hazard of them with some food borne pathogens. The bacteriological examination of samples collected from, Benha city, centers and villages revealed that, the mean value of APC, Psychrotrophic, Enterobacteriaceae, Coliform and Staphylococcus counts in frozen beef burger samples collected from Benha city were $2.97 \times 10^4 \pm 0.15 \times 10^4$; $1.76 \times 10^4 \pm 0.11 \times 10^4$; $1.35 \times 10^2 \pm 0.11 \times 10^2$; $0.73 \times 10^2 \pm 0.18 \times 10^2$ and $1.34 \times 10^2 \pm 0.09 \times 10^2$, respectively; for samples collected from different centers were $4.74 \times 10^4 \pm 0.17 \times 10^4$; $2.32 \times 10^4 \pm 0.10 \times 10^4$; $1.74 \times 10^2 \pm 0.10 \times 10^2$; $1.29 \times 10^2 \pm 0.09 \times 10^2$ and $1.88 \times 10^2 \pm 0.10 \times 10^2$, respectively; for samples collected from different villages were $7.91 \times 10^4 \pm 0.16 \times 10^4$; $5.21 \times 10^4 \pm 0.19 \times 10^4$; $3.36 \times 10^2 \pm 0.17 \times 10^2$; $2.23 \times 10^2 \pm 0.13 \times 10^2$ and $2.38 \times 10^2 \pm 0.10 \times 10^2$, respectively. 14 isolates of *E. coli* were isolated from examined frozen beef burger samples collected from different localities (Benha city, centers and villages) represented as 2(5.7%) from samples of Benha city with serotypes one O55:K59(B5) and one O125:K59(B5); 5 (14.3%) from samples of centers with serotypes 2 O55:K59(B5), 2 O125:K59(B5) and one O126:K71 (B16) and 7 (20.0%) from samples of villages with serotypes 3 O55:K59(B5), 2 O125:K59(B5), one O111:K58(B9) and one O126:K71(B16). In addition, 19 isolates of Coagulase positive *S. aureus* were isolated, 3 (8.6%) from samples of Benha city; 6 (17.1%) from samples of centers and 10 (28.6%) from samples of villages. SET- RPLA test revealed that, 6 strains out of 10 randomly examined strains (60.0%) were enterotoxigenic and classified according to type of toxin into (3A; 1C, 2A and C). *Ps. aeruginosa* strains were the only species isolated from examined samples and three beef burger samples collected from different villages were only positive for their isolation. Moreover, the present study failed to detect *Aeromoneus* species and *Salmonella serovars* from all examined frozen beef burger samples.

Keywords: *Beef Burgers; Bacteriological Evaluation; SET- RPLA Test; E. coli; S. aureus; Ps. aeruginosa*

Introduction

Beef burgers are important and popular food items of highly nutritious and highly desirable foods for human being, on the other hand, they are considered as an ideal culture medium for growth of many organisms because of the high moisture, the high percentage of nitrogenous compounds, plentiful supply of minerals, some fermentable carbohydrates (glycogen) and of a favorable pH for most microorganisms resulting in their spoilage, economic losses, foodborne infections in human and health risk [1,2].

Microbiological aspects are a useful way to determine the safety and quality of meat product and they may be contaminated during processing from the hands, workers clothes, knives, the hide, the gut or from the environment and transportation resulting in an inferior or even unfit quality for human consumption [3,4]. The most important bacterial pathogens in beef meat and meat products that are responsible for food-borne infections include *E. coli*, *Salmonellae*, coagulase positive *S. aureus* and *Pseudomonas* [2,5,6].

The bacterial contamination and hygienic measures during meat production and bad storage conditions for frozen meat products can be measured using the aerobic plate count, total psychrotroph counts and three Gram - negative indicator groups viz: Total Enterobacteriaceae, total Coliforms and *Escherichia coli* biotype 1, which is the most important indicator for faecal contamination [6-8]. *E. coli* is used as surrogate indicator, because its presence in food generally indicates direct and in direct fecal contamination [9]. It is commonly non-virulent but some strains have adopted pathogenic or toxigenic virulence factors that make them virulent to human and animals. It has become recognized as a serious food borne pathogen and has been associated with numerous out breaks of disease resulting from contaminated beef and meat products, including bacteremia, urinary tract infections, neonatal meningitis, pneumonia, deep surgical wound infections, endovascular infections, vertebral osteomyelitis, and septicemia [10-12]. Infections with *Salmonellae* and coagulase positive *S. aureus*, are the causative agents of two thirds of food-borne disease outbreaks causing gastroenteritis and rarely acquired directly from raw meat but mostly occurs either due to excessive handling or contamination during or after cooking of meat and meat products [13,14].

The *Staphylococci* enterotoxins causing food poisoning are produced by about one-third of coagulase positive *S. aureus* strains and growth of enterotoxigenic strains of *S. aureus* to population of at least 10^5 cfu/g of food is generally considered necessary for production of sufficient amount of enterotoxins to induce food intoxication, that characterized by symptoms including nausea, vomiting, abdominal cramps and diarrhea lasting from 24 to 48h and the complete recovery usually occurs within 1 - 3 days [15]. Recent food surveys confirmed that *Aeromonas* spp. and *Pseudomonas* spp. were considered as re- immerging enteric pathogens that responsible for several food borne illness and outbreaks [5,16]. As the level of contamination of frozen beef burger with different food-borne pathogens constitutes serious problems for consumers, so, the present study was conducted to study the bacteriological aspects, the safety and quality of frozen beef burger at different localities in Kaliobia Governorate.

Material and Methods

Samples collection

A total of 105 random samples of frozen beef burgers, were collected from different localities (Benha city, centers and villages with 35 for each locality) at Kaliobia Governorate. Each sample was kept in a separate sterile plastic bag and put in an icebox then transferred to the laboratory under complete aseptic conditions without undue delay and examined bacteriologically to evaluate the bacterial quality and the hygienic health hazard of them with some food borne pathogens.

Bacteriological examination

1. Preparation of samples [17].
2. Determination of Aerobic Plate Count (APC)/gram, using the standard plate count following [18].
3. Determination of Total Psychrotrophic count [17].
4. Determination of Total Enterobacteriaceae count using the surface plating method of ICMSF, 1996 [19] using Violet Red Bile Glucose agar medium (VRBG). The plates were incubated at 37°C for 24 hours. All purple colonies were then counted and the total number of colonies was determined. Hence, the Enterobacteriaceae count/g was calculated and recorded.
5. Total Coliform count using the surface plating method of ICMSF, 1996 [19] using Violet Red Bile agar medium.
6. Isolation and identification of *E. coli* following [20]: Typical *E. coli* colonies (pink - orange colonies) were picked up for identification morphologically by Gram stain; biochemically, serologically by slide agglutination test (using *E. coli* antisera “SEIKEN” Set 1, consists of 8 polyvalent and 43 (OK) antisera of DENKA SEIKEN Co. LTD. Tokyo, Japan) following [21,22].

7. Determination of Total Staphylococci Count following [19]. Isolation of *S. aureus* using Baird-Parker Agar Plates. Suspected colonies were picked up onto slants of nutrient agar for further purification then identified morphologically by Gram-stain; biochemically and coagulase activities according to ICMSF (1996) [19] and Quinn., *et al* [22].

   Detection of Enterotoxins producing isolates by SET- RPLA technique [23].
8. Isolation and identification of Pseudomonas species following [22,24].
9. Isolation of Aeromonas species following [22,25].
10. Isolation and identification of *Salmonella* following [26]. Suspected *Salmonella* colonies that appeared as red with black centers on XLD agar and pink on Brilliant Green agar were identified morphologically by Gram-stain and biochemically according to [22].
11. Data obtained were analyzed according to Snedecor and Cochran [27] using the computer software program [28].

Results

The results of bacteriological examination of frozen beef burger samples collected from different areas at Kaliobia Governorate (Benha city, different centers and different villages) are presented in tables 1-8.

Total aerobic bacterial count (APC)

Sample area	Positive		Min.	Max.	Mean ±SEM**
	No.	%*			
Benha city	35	100.0	0.9×10^4	4.9×10^4	$2.97 \times 10^4 \pm 0.15 \times 10^{4c}$
Centers	35	100.0	2.5×10^4	6.4×10^4	$4.74 \times 10^4 \pm 0.17 \times 10^{4b}$
Villages	35	100.0	4.8×10^4	9.9×10^4	$7.91 \times 10^4 \pm 0.16 \times 10^{4a}$

Table 1: *Aerobic plate counts/gm (APC) in the examined samples of frozen beef burger (n = 35 for each sample).*

**Percentage in relation to total number of sample in each row.*
***Standard error of mean*

Total Psychrotrophic counts

Sample area	Negative		Positive		Min.	Max.	Mean ± SEM**
	No.	%*	No.	%*			
Benha city	6	17.1	29	82.9	0.5×10^4	3.2×10^4	$1.76 \times 10^4 \pm 0.11 \times 1^{04}c$
Centers	3	8.6	32	91.4	1.2×10^4	3.4×10^4	$2.32 \times 10^4 \pm 0.10 \times 10^{4b}$
Villages	0	0.0	35	100.0	3.2×10^4	7.4×10^4	$5.21 \times 10^4 \pm 0.19 \times 10^{4a}$

Table 2: *Total Psychrotrophic counts/gm in the examined samples of frozen beef burger (n = 35 for each sample).*

**Percentage in relation to total number of sample in each row.*
***Standard error of mean*

Total Enterobacteriaceae count

Sample area	Negative		Positive		Min.	Max.	Mean ±SEM**
	No.	%*	No.	%*			
Benha city	16	45.7	19	54.3	0.8×10^2	2.5×10^2	$1.35 \times 10^2 \pm 0.11 \times 10^{2b}$
Centers	10	28.6	25	71.4	1.0×10^2	2.8×10^2	$1.74 \times 10^2 \pm 0.10 \times 10^{2b}$
Villages	2	5.7	33	94.3	1.9×10^2	4.9×10^2	$3.36 \times 10^2 \pm 0.17 \times 10^{2a}$

Table 3: *Enterobacteriaceae counts/gm in the examined samples of frozen beef burger (n = 35 for each sample).*

**Percentage in relation to total number of sample in each row.*
***Standard error of mean*

Total Coliform count

Sample area	Negative		Positive		Min.	Max.	Mean ± SEM**
	No.	%*	No.	%*			
Benha city	27	77.1	8	22.9	0.3×10^2	1.8×10^2	$0.73 \times 10^2 \pm 0.18 \times 10^{2c}$
Centers	14	40.0	21	60.0	0.7×10^2	2.2×10^2	$1.29 \times 10^2 \pm 0.09 \times 10^{2b}$
Villages	6	17.1	29	82.9	1.1×10^2	3.6×10^2	$2.23 \times 10^2 \pm 0.13 \times 10^{2a}$

Table 4: *Coliforms counts/gm in the examined samples of frozen beef burger (n = 35 for each sample).*

**Percentage in relation to total number of sample in each row.*

***Standard error of mean*

Isolation of *E. coli*

Sample area	No.	Positive		No. of accepted samples**	No. of non-accepted samples**
		No.	%*		
Benha city	35	2	5.7	33	2
Centers	35	5	14.3	30	5
Villages	35	7	20.0	28	7
Total	105	14	13.3	91	14

Table 5: *Incidence of E. coli in examined samples of frozen beef burger (n = 35 for each sample).*

**Percentage in relation to total number of sample in each row.*

***Accepted and non- accepted samples according to (EEC, 2005).*

Serotyping of isolated *E. coli*

Sample area	Benha city		Centers		Villages		Strain characteristic
E.coli serotype	No.	%*	No.	%*	No.	%*	
O55:K59(B5)	1	2.86	2	5.71	3	8.57	EPEC
O125:K59(B5)	1	2.86	2	5.71	2	5.71	ETEC(EPEC)
O111:K58(B9)	0	0.0	0	0.0	1	2.86	EHEC
O126:K71(B16)	0	0.0	1	2.86	1	2.86	EPEC
Total	2	5.71	5	14.28	7	20.00	-

Table 6: *Incidence and serotyping of E. coli isolated from positive samples of frozen beef burger (n = 35 for each sample).*

**Percentage in relation to total number of each sample (35).*

EPEC: Enteropathogenic E. coli

ETEC: Enterotoxigenic E. coli EHEC: Enterohaemorrhagic E. coli

Staphylococcus species

Total *Staphylococcus* count

Sample area	Negative		Positive		Min.	Max.	Mean ±SEM**
	No.	%*	No.	%*			
Benha city	13	37.1	22	62.9	0.6×10^2	2.4×10^2	$1.34 \times 10^2 \pm 0.09 \times 10^{2c}$
Centers	8	22.9	27	77.1	1.0×10^2	2.9×10^2	$1.88 \times 10^2 \pm 0.10 \times 10^{2b}$
Villages	4	11.4	31	88.6	1.2×10^2	3.8×10^2	$2.38 \times 10^2 \pm 0.10 \times 10^{2a}$

Table 7: *Staphylococci counts/gm in the examined samples of frozen beef burger (n = 35 for each sample).*

**Percentage in relation to total number of sample in each row.*
***Standard error of mean*

Isolation of Coagulase Positive *S. aureus*

Sample area	No.	Positive		No. of accepted samples**	No. of non-accepted samples**
		No.	%*		
Benha city	35	3	8.6	32	3
Centers	35	6	17.1	29	6
Villages	35	10	28.6	25	10
Total	105	19	18.1	86	19

Table 8: *Incidence of Coagulase Positive S. aureus in examined samples of frozen beef burger (n = 35 for each sample).*

**Percentage in relation to total number of sample in each row.*
***Accepted and non- accepted samples according to (EEC, 2005) in relation to the isolation of Coagulase Positive S. aureus.*

Results of Enterotoxins producing *S. aureus* strains

The results of SET-RPLA test revealed that, 6 strains out of 10 random examined strains (60.0%) were enterotoxigenic and classified according to type of toxin into (3A;1C, 2A and C).

Isolation of *Pseudomonas* species

Ps. aeruginosa strains were the only species isolated from examined samples. Only three beef burger samples collected from different villages were positive for *Ps. aeruginosa* isolation, meanwhile, they failed to be detected in all examined samples of frozen beef burger collected from Benha city and different centers at Kaliobia Governorate.

Isolation of *Aeromoneus* species

Aeromoneus species were failed to be detected in all examined samples of frozen beef burger.

Isolation of *Salmonella* species

Salmonella serovars were failed to be detected in all examined samples of frozen beef burger.

Discussion

Beef burgers are important and popular food items of highly nutritious and highly desirable foods for human being, but they are considered as an ideal culture medium for growth of many microorganisms as *E. coli; Salmonella; S. aureus; Pseudomonas; Micrococcus; lacto-*

bacillus and *Aeromoneus* resulting in their spoilage, economic losses, foodborne infections in human and health risk [1,6,29]. Therefore, the present study was carried out on frozen beef burger at Kaliobia Governorate to evaluate the bacterial quality and the hygienic health hazard of them with some food borne pathogens.

Total aerobic bacterial count (APC)

The total aerobic bacterial count can be used as indicator of bad hygiene during food processing and bad storage conditions that can lead to toxins production and pathogens proliferation [8]. Limits suggested for total aerobic bacterial count in beef burger is 10^5 microbes/g [30].The data shown in table 1 revealed that, the minimum and the maximum aerobic plate counts (APC) in the examined frozen beef burger samples collected from different localities (Benha city, centers and villages) were ranged from 0.9×10^4 to 4.9×10^4; 2.5×10^4 to 6.4×10^4 and 4.8×10^4 to 9.9×10^4 respectively, with a mean value of $2.97 \times 10^4 \pm 0.15 \times 10^4$; $4.74 \times 10^4 \pm 0.17 \times 10^4$ and $7.91 \times 10^4 \pm 0.16 \times 10^4$, respectively. All examined samples (100%) contained microorganisms. However, the counts were considered satisfactory, as these results were lower than those suggested by EEC [30]. Nearly similar counts were recorded by Zalouk-Enas [31]; Mousa., *et al.* [32]; and Hamed., *et al* [6].

Total Psychrotrophic counts

The results in table 2 appeared that, the minimum and the maximum Psychrotrophic count in the examined beef burger samples collected from different localities (Benha city, centers and villages) were ranged from 0.5×10^4 to 3.2×10^4; 1.2×10^4 to 3.4×10^4 and 3.2×10^4 to 7.4×10^4 respectively, with a mean value of $1.76 \times 10^4 \pm 0.11 \times 10^4$; $2.32 \times 10^4 \pm 0.10 \times 10^4$ and $5.21 \times 10^4 \pm 0.19 \times 10^4$, respectively. As all positive samples of frozen beef burger collected from different areas were lower than 10^5, so all samples were accepted following EEC [30]. These results of were agree with those of Karaboz and Dincer [33].

Total Enterobacteriaceae count

Enterobacteriaceae have an epidemiological importance and the presence of them in meat indicates a microbial proliferation, as some of their members are pathogenic and may cause serious infections and food poisoning outbreaks to human being [34]. The results in table 3 appeared that, the minimum and the maximum Enterobacteriaceae count in the examined frozen beef burger samples collected from different localities (Benha city, centers and villages) were ranged from 0.8×10^2 to 2.5×10^2; 1.0×10^2 to 2.8×10^2 and 1.9×10^2 to 4.9×10^2 respectively, with a mean value of $1.35 \times 10^2 \pm 0.11 \times 10^2$; $1.74 \times 10^2 \pm 0.10 \times 10^2$ and $3.36 \times 10^2 \pm 0.17 \times 10^2$, respectively. These results of were agree with those of Stagnitta., *et al.* [35] and Zalouk-Enas [31].

Total Coliform count

The presence of coliforms in food indicates poor hygienic standards. Data presented in table 4 showed that, the minimum and the maximum Coliform count in the examined frozen beef burger samples collected from different localities (Benha city, centers and villages) were ranged from 0.3×10^2 to 1.8×10^2; 0.7×10^2 to 2.2×10^2 and 1.1×10^2 to 3.6×10^2 respectively, with a mean value of $0.73 \times 10^2 \pm 0.18 \times 10^2$; $1.29 \times 10^2 \pm 0.09 \times 10^2$ and $2.23 \times 10^2 \pm 0.13 \times 10^2$, respectively. These results came in parallel with those of Mousa., *et al.* [32] and Hamed., *et al* [6].

Isolation of *E. coli*

The recovery of *E. coli* from meat samples indicates fecal contamination and implies that other pathogens of fecal origin may be present. The increased incidence of *E. coli* in the examined samples may be due to mishandling during production, processing and distribution or to the use of contaminated water during evisceration and slaughtering [36,37]. The results in tables (5 and 6) revealed that, 14 isolates of *E. coli* were isolated from examined frozen beef burger samples collected from different localities (Benha city, centers and villages) represented as 2 (5.7%) from samples of Benha city with serotypes one O55:K59(B5) and one O125:K59(B5); 5 (14.3%) from samples of centers with serotypes 2 O55:K59(B5), 2 O125:K59(B5) and one O126:K71(B16) and 7 (20.0%) from samples of villages with serotypes 3

O55:K59(B5), 2 O125:K59(B5), one O111:K58(B9) and one O126:K71(B16), also, 91 samples out of 105 ones were accepted as they were free from *E. coli* isolates according to [30]. Nearly similar results were obtained by Maarouf and Nassif-Marionette [38]; Mansour [39]; Ezzat., *et al.* [40]; Mohammed., *et al.* [41] and Abd El-Tawab., *et al* [42]. Meanwhile, these results were disagreed with those of Zaki-Eman [43] and Ramadan [44] who isolated *E. coli* from frozen beef burger samples with high incidence**.** Also, disagreed with Wehab and Hegazy [45] and Hamed., *et al.* [6] who failed to isolate *E. coli* from beef burger samples. Moreover, the same serotypes of *E. coli* were previously isolated by Maarouf and Nassif-Marionette [38]; Mansour [39]; Mohammed., *et al.* [41]; Shawish [46] and Tarabees., *et al* [47].

Total *Staphylococcus* count

The obtained results in table 7 revealed that, the minimum and the maximum Staphylococcus count in the examined frozen beef burger samples collected from different localities (Benha city, centers and villages) were ranged from 0.6×10^2 to 2.4×10^2; 1.0×10^2 to 2.9×10^2 and 1.2×10^2 to 3.8×10^2 respectively, with a mean value of $1.34 \times 10^2 \pm 0.09 \times 10^2$; $1.88 \times 10^2 \pm 0.10 \times 10^2$ and $2.38 \times 10^2 \pm 0.10 \times 10^2$, respectively. These counts came in agreement with El-Maghraby-Marwa [48] and Ahmed- Alyaa [49]. Meanwhile, the results disagreed with those of Moharum [50] and Saad., *et al.* [51] who reported higher Staphylococcus counts in examined frozen beef burger samples.

Moreover, the statistical results revealed that, samples collected from different villages showed a significant ($P \leq 0.05$) increase of APC; total Psychrotrophic counts; Enterobacteriaceae counts; Coliforms counts and Staphylococci counts when compared with other samples. This may be due to the combination of the low quality of beef burger sold; poor manufacturing processes; inadequate cleaning and disinfection of both equipment and surfaces or poor personal hygiene; use of untrained personnel and long storage periods with periodical cutting of electrics or using inconstant power of electric supply with fuel powered generating sets, that leading to frequent thawing and freezing of products in villages resulting in an inferior or even unfit quality for human consumption [3,4,52].

Isolation of Coagulase Positive *S. aureus*

The results obtained in table 8 revealed that, 19 isolates of Coagulase positive *S. aureus* were isolated from examined frozen beef burger samples represented as 3 (8.6%) from samples of Benha city; 6 (17.1%) from samples of centers and 10 (28.6%) from samples of villages. Moreover, 86 samples out of 105 ones were accepted, as they were free from Coagulase Positive *S. aureus* isolates according to [30]. These results came in accordance with those obtained by Abd El-Tawab., *et al*. [42]; Djoulde., *et al.* [53]; Hamed., *et al.* [6]; Tarabees., *et al.* [47] and Nadim-Samaa [54]. Meanwhile, these results were disagreed with those of Mousa., *et al.* [32] who isolated *S. aureus* from frozen beef burger samples with high incidence. Also, disagreed with Wehab and Hegazy [45] who failed to isolate *S. aureus* from beef burger samples. The presence of *S. aureus* in meat and its products indicates poor hygiene of meat handlers as well as lack of sterilization of utensils and they grow without pronounced change in odour or taste in the products and producing heat stable enterotoxins which lead to food poisoning with severe diarrhoea and gastroenteritis among consumers [55]. Regarding to the results of SET- RPLA test table 10 revealed that, 6 strains out of 10 randomly examined strains (60.0%) were enterotoxigenic and classified according to type of toxin into (3A; 1C, 2A and C). This result nearly similar to that recorded by [40,42,56] who found enterotoxin A; C and A and C in beef meat and meat products.

Isolation of *Pseudomonas* species

Pseudomonas spp. are the most important spoilage organisms associated with meat and meat products, as the presence of them in meat and meat products lead to unsafe food [5]. The results of the present study revealed that, *Ps. aeruginosa* strains were the only species isolated from examined samples, where, only three beef burger samples collected from different villages were positive for *Ps. aeruginosa* isolation, meanwhile, they failed to be detected in all examined samples of frozen beef burger collected from Benha city and different centers at Kaliobia Governorate. Similar results for *Ps. aeruginosa* strains isolation from frozen beef burger samples were recorded by El-Shopary [57].

Isolation of *Aeromoneus* species

The present study failed to detect Aeromoneus species from all examined frozen beef burger samples.

Isolation of *Salmonella* species

The present study failed to detect *Salmonella serovars* from all examined frozen beef burger samples. These results were agreed with those recorded by Datta., *et al* [12]. Meanwhile, disagreed with those of Mousa., *et al*. [32]; Abd El-Tawab., *et al*. [42] and Hamed., *et al*. [6] who isolated *Salmonella* from frozen beef burger samples.

Finally, the present study proved that frozen beef burgers are considered public health hazard and the presence of aerobic bacteria; Enterobacteriaceae; coliforms; *E. coli*; *Staphylococci* mainly Coagulase Positive *S. aureus; Ps. aeruginosa* and *Psychrotrophic* bacteria may be due to mishandling and the negligence of hygienic aspects. Therefore, it was concluded that these pathogens are meat-borne pathogens of public health important.

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