Abstract

Introduction: Antimicrobial resistance is defined as the resistance of an antimicrobial agent that is used to be effective against microbes but no longer works. Antimicrobial resistance is becoming a serious threat to global health as it is increasing worldwide requiring an integrated global action.

Objective: To assess the prevalence and identify antimicrobial resistance to commonly used drugs in relation to different types of samples from July to December, 2016 at National Health Laboratory, Asmara, Eritrea.

Methods: Retrospective documentary review study of laboratory results at National Health Laboratory, department of microbiology was applied to determine the pattern of antimicrobial resistance for bacterial isolates. All samples that brought to the microbiology laboratory were included in the study. Those samples that showed growth of isolates underwent for culture and sensitivity test against the commonly uses antimicrobial drugs. Statistical analysis of data was done using SPSS version 20.

Results: Out of 398 total samples, 149 samples had shown bacterial growth. Of these 149 samples: 48 were tested for *E. Coli* and found to be resistant to Ampicillin (42 (87.5%)) and sensitive to chloramphenicol (35 (72.9%)); 20 were tested for *Klebsiella* spp. found to be resistant to Ampicillin (15 (75%)) and sensitive to Chloramphenicol in (16 (80%)); 18 tested for *Citrobacter* spp. found to be resistant to Ampicillin in (18 (100%)) and sensitive for Amikacin in (11 (61%)); 11 tested for *Pseudomonas* spp found to be resistant to majority of the drugs Ampicillin, Nalidixic acid, and Tetracycline, (9(81.8%) each), Cephalexin and Cefpazidime (8 (72.7%) each), and Ceftriaxone (7(63.6%)); but they were found to be sensitive to Amikacin and Ciprofloxacin in 8 (72.7%) and 7 (63.6%), respectively. 8 of the samples tested for *Proteus* spp. were found to be resistant to Nitrofurantoin and Tetracycline (8 (100%) each), where as they were observed to be sensitive to Amikacin 5 (62.5%). 6 tested for *Salmonella* spp. and were also found to be sensitive to Amikacin and Ciprofloxacin in (4 (66.7%), each), whereas resistant to Ampicillin 6 (100%). Three samples were tested for *Staphylococcus aureus* and found to be resistant to most of the drugs; Nitrofurantoin 3 (100%), and Gentamycin, Vancomycin, Oxacillin and Penicillin (2 (66.7%) each); 35 samples were also tested for other isolates that include; *Aeromonas Spp.*, *Listeria Domsella*, *Candida Spp.*, *Streptococcus Viridians*, Klavera Spp., *Providencia Spp.*, *Enterobacter Cloacae*, *Proteus Vulgaris*, *Chrysosmonas Lutasa*, *Morganella Morganii*, Neisseria Meningitidis, *Acinetobacter Spp.* and *Pasteurella Maltocida*; and showed a resistance to Cephalexin and Tetracycline (28 (80.1%), each), Ampicillin 27 (77.1%), Nalidixic Acid 26 (74.3%) and Co-trimoxazole 21 (60%).

Discussion and Conclusion: The study results showed that most of the bacterial isolates were sensitive to Chloramphenicol Amikacin, Ciprofloxacin, Ceftriaxone and Gentamycin; whereas they were resistant to Cephalexin, Ampicillin, Cefazidime, Nitrofurantoin, Co-trimoxazole, Nalidixic acid and Tetracycline. Therefore, the study recommends the practice of rational drug use to be permissible and continuous surveillance for antimicrobial drug sensitivity test to be done in order to assure appropriate drug administration for treating disease and reducing the emergence of new resistant strains of bacteria.

Keywords: Culture; Sensitivity; Bacterial isolates; Antimicrobial growth and resistance

Abbreviation: AMR: Antimicrobial Resistance; CLSI: Clinical laboratory Standards Institute; CSF: Cerebro-spinal fluid; ENF: Eritrean National Formulary; ENLM: Eritrean National List of Medicine; LMICs: Low-income and Middle-Income Countries;
NHL: National Health Laboratory; SPSS: Statistical Package for Social Science

Introduction

Antimicrobials are natural, semi synthetic or synthetic substances that are capable of killing or inhibiting the growth of microbes [1]. Antimicrobial resistance (AMR) simply means that the antimicrobial drugs that used to be effective against a particular microbe no longer work because of the change in the biological make-up of the microbe that makes it resistant to treatment [2,3]. The problem occurs naturally, or when an infection is treated with an antimicrobial that kills only some of the microbes and making others resistant for the treatment by leaving to survive and multiply in the environment [2]. In general there are many reasons for an increase of antimicrobial resistance that include: increasing consumption of antibiotics worldwide, as a result of treatment or diagnosis uncertainty physicians prescription of more antibiotics, significant access of antimicrobials agents in the use of as prophylaxis and self-administered treatment of infection [4,5], noncompliance with infection-control practice, increased duration of hospital stay, co-morbidities in hospitalized patients, frequent use of invasive devices and catheters, weak infection-control practices, transfer of colonized patients from hospital to hospital, grouping of patients in long-term-care facilities, use of antibiotic for animals and household tasks, and increasing national and international travel [6]. The pattern of antimicrobial resistance differs from time to time, country to country and among hospitals within the same country. As a result the health care cost for patients with resistant infections is higher than non-resistant infections, due to longer duration of illness, additional tests and use of more expensive drugs [7]. Over time, more and more of the resistant microbes remain in the environment, eventually leading to the emergence of new strains of disease-causing microbes that are partially or fully resistant to antimicrobial treatment [8,9]. The level of antibiotic resistance depends on: the population of organisms that spontaneously acquire resistance to selective pressure from antibiotic use, rate of introduction of those resistant organisms into the health care settings, and the proportion of spread from person to person [10].

Antimicrobial resistance is one of the serious problems due to new drug-resistant bacterial infections that are becoming more prevalent and becoming threat to the world public health care services and causing financial challenges [11,12] (Coast, Smith & Superbugs 1996; Smith 1999; Kohanski et al. 2010). Antimicrobial resistance is a global issue rather than a local or national. Data of regional and national antimicrobial resistance and trends of clinically important bacterial isolates are helpful for physicians in making decisions concerning the appropriate empirical treatment of various infections.

Therefore, this study was designed to explore the prevalence of antimicrobial resistance to the most common drugs that are used in the country for treating patients with different diagnosis of disease by doing culture and sensitivity test for different types of human samples that are collected at the National Health Laboratory (NHL) of Asmara, Eritrea. The study included the drugs that are listed in the Eritrean National Formulary [13] and Eritrean National List of Medicine [14], that are available in the country as per to the government policy in matching to the Clinical Laboratory Standard Institute [15] Guidelines; in order to provide a baseline information of sensitivity to these drugs. This study will have a great contribution for further research and will help also for health planners to design a strategy for effective and proper antimicrobial drug use.

Objectives

To assess and determine the prevalence of antimicrobial resistance to eighteen commonly used drugs in relation to different types of samples having various types of bacterial pathogen isolates from July 1st to December 31st, 2016 at National Health Laboratory, Asmara, Eritrea.

Methods and Materials

Study setting and design

A retrospective cross-sectional study was conducted at National Health Laboratory (NHL), Asmara, Eritrea. The study was done based on retrospective record review of patients from July 1st to December 31st, 2016; on antimicrobial susceptibility tests performed on bacterial isolates in the National Health Laboratory Department of Microbiology Asmara, Eritrea.

Study participants and study period

All samples that were brought to the study area were included. Those samples that showed growth of bacterial isolates that underwent sensitivity test against the commonly used antimicrobial drugs based on the ENF and ENLM that correspond with the CLSI as well.

Data collection

Data related to the variables of interest were collected, based on the common tests that were performed in the Microbiology Laboratory. Primarily qualitative data reflecting socio-demographic and resistance level of microbes (resistant, intermediate, or susceptible) was collected. The collected data was entered into an Excel. Variables of interest included the first isolate from each specimen of patient such as ascitic fluid, pleural fluid, blood, Cerebro – spinal fluid (CSF), synovial fluid, nasal swab, sputum, seminal fluid, urine, urethral discharge, and vaginal discharge, stool, and pus. The specimens were analyzed based on the isolate and with different antibiotics susceptibility profiles.

Data processing and analysis

The study used SPSS version 20 for statistical analysis of the data collected.

Ethical consideration

The study obtained permission from Ministry of Health ethical and research committee, and National Health Laboratory and all records with in the study period were checked. The procedure of the study was very confidential in that the records were checked by the researchers only.
Results

The number of specimens that were collected at National Health Laboratory, Asmara, Eritrea, from July 1st to December 31st, 2016 included a total of four hundreds of different types. Those samples that were included in the review were tested by using Mueller Hintone Agar for culture and sensitivity test of the antimicrobials. Therefore, out of these four hundred specimens that under went for culture and sensitivity test the number of adults was higher 67.75 percent (n=271) as compared to the number of children 32.25 percent (n=129). The number of females (n=210) was also higher than that of males (n=190) (Table 1).

Table 1: Demographic characteristics of patients for culture and sensitivity test.

| Gender          | Children (<18 Years) | Adults (> 18 Years) | Total |
|-----------------|----------------------|---------------------|-------|
| Male            | 50 (26.3%)           | 140 (73.7%)         | 190   |
| Female          | 79 (37.6%)           | 131 (62.4%)         | 210   |
| Total Patients  | 129 (32.2%)          | 271 (67.75%)        | 400   |
| Mean age in years (standard deviation) | 4.43 (4.8) | 42.35 (16.2) | 30.12 (22.4) |

Sample types with presence or absence of growth

The specimen were categorized based on their type as ascitic fluid, pleural fluid, blood, CSF, synovial fluid, nasal swab, sputum, seminal fluid, urine, urethral discharge, vaginal discharge, stool, and pus.

The most frequent specimen with 60.05 Percent (n=239) was urine and the least were synovial fluid and sputum with 0.25 percent (n=1) each. In majority of the samples growth of bacteria was not observed having general result of non-growth 62.6 percent (n=249) as compared to 37.4 percent (n=149) growth (Table 2).

Table 2: Frequency of samples and distribution of presence or absence of growth of various isolates.

| Sample Type     | Frequency of Presence or Absence of Growth |
|-----------------|------------------------------------------|
|                 | No (%) | Yes (%) | Total Samples |
| Ascitic Fluid   | 3 (75%) | 1 (25%) | 4 (%)         |
| Pleural Fluid   | 4 (100%) | 0 (0%)  | 4 (%)         |
| Blood           | 54 (96.4%) | 2 (3.6%) | 56 (14.07%)   |
| CSF             | 16 (88.9%) | 2 (11.1%) | 18 (4.52%)    |
| Synovial Fluid  | 1 (100%) | 0 (0%)  | 1 (0.25%)     |

Antimicrobials susceptibility pattern of isolates

The total samples (n=149) that showed growth were tested for sensitivity against different drugs. All (eighteen) the commonly used antibiotics that went through culture and sensitivity test of different samples for various pathogens in the study period are abbreviated as per to the Clinical laboratory Standards Institute (CLSI), (Table 3 & 4). According to the results out of forty eight samples that were tested for *E. Coli*, it was found to be resistant to Ampicillin (42 (87.5%)) and sensitive to chloramphenicol (35(72.9%)).

Twenty samples showing growth of *Klebsiella spp.* were also tested and it was found to be resistant to Ampicillin (15(75%)) and sensitive to Chloramphenicol in (16(80%)). Out of eighteen samples of *Citrobacter spp.* a resistance to Ampicillin in (18(100 %)) and sensitivity to Amikacin in (11(61%)) (Table 3). Out of eleven samples, *Pseudomonas spp.* were also found to be resistant to the majority of the drugs; Ampicillin, Nalidixic acid and Tetracycline, (9(81.8%) each), *E. Coli* and *Cefazidime* (8 (72.7%) each), and *Ceftriaxone* (7(63.6%)); but they were found to be sensitive to Amikacin and Ciprofloxacin for 8 (72.7%) and 7(63.6%), respectively (Table 3 & 4). Eight samples had culture and sensitivity test for *Proteus spp.* in which Nitrofurantoin and Tetracycline were found to be resistant (8(100%) each), whereas sensitive to Amikacin 5(62.5%) (Table 3). Out of six samples that showed growth for *Salmonella spp.* sensitivity was observed to Amikacin and Ciprofloxacin in (4(66.7%) each), whereas resistant to Ampicillin 6(100%). Out of three samples *Staphylococcus aureus* was observed to be resistant to most of the drugs; Nitrofurantoin 3(100%), and Gentamycin, Vancomycin, Oxacillin and Penicillin in (2(66.7%), each) (Table 3 & 4).There were also other isolates that include: *Aeromonas Spp.*, *Listeria Domella*, *Candido Spp.*, *Streptococcus Viridans*, *Kluvera Spp.*, *Providencia Spp.*, *Enterohector Cloacae*, *Proteus Vulgaris*, *Chryssemonas Lutasa*, *Morganella Morganii*, *Neisseria Meningitidis*, *Acinetobacter Spp.* and *Pasteurella Multocida* from the samples that were tested for the antimicrobial susceptibility and they were observed to be resistant to Cephalexin and Tetracycline (28(80.1%), each). Ampicillin 27(77.1%) Nalidixic acid 26(74.3%) and Co-trimoxazole 21(60%) (Table 3 & 4).
Table 3: Antibiotic susceptibility pattern of twelve most frequent bacterial isolates.

| Bacterial Isolate | TS | Pat | Antimicrobial Agents, n (%) |
|-------------------|----|-----|-----------------------------|
|                   |    |     | AK  | AMX | CN  | CAZ | CRO | CIP | COT | GM  | NIT | NA  | TE  | EC  |
| E. Coli           | 48 |     |     |     |     |     |     |     |     | 4   |     | 0   | 0   | 0   |
|                   |    |     | 2(4.2%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 2(4.2%) | 1(2.1%) | 1(2.1%) |
| Klebsiella spp.   | 20 |     |     |     |     |     |     |     |     |     |     |     |     |
|                   |    |     | 1(5%) | 1(5%) | 2(10%) | 0(0%) | 0(0%) | 0(0%) | 1(5%) | 0(0%) | 0(0%) | 3(15%) |
| Citrobacter spp.  | 12 |     |     |     |     |     |     |     |     |     |     |     |     |
|                   |    |     | 2(16.7%) | 0(0%) | 3(25%) | 1(8.3%) | 0(0%) | 0(0%) | 1(8.3%) | 0(0%) | 0(0%) | 0(0%) | 1(6.6%) |
| Pseudomonas spp.  | 11 |     |     |     |     |     |     |     |     |     |     |     |     |
|                   |    |     | 1(9.1%) | 0(0%) | 0(0%) | 1(9.1%) | 0(0%) | 1(9.1%) | 0(0%) | 1(9.1%) | 0(0%) | 0(0%) | 0(0%) |
| Proteus spp.      | 8  |     |     |     |     |     |     |     |     |     |     |     |     |
|                   |    |     | 1(25%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 1(25%) | 0(0%) | 0(0%) |
| Salmonella spp.   | 6  |     |     |     |     |     |     |     |     |     |     |     |     |
|                   |    |     | 1(16.7%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 1(16.7%) | 0(0%) | 0(0%) |
| S. aureus         | 3  |     |     |     |     |     |     |     |     |     |     |     |     |
|                   |    |     | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 1(33.3%) |
| Other isolates*   | 35 |     |     |     |     |     |     |     |     |     |     |     |     |
|                   |    |     | 1(2.1%) | 0(0%) | 3(100%) | 1(33.3%) | 0(0%) | 1(33.3%) | 0(0%) | 0(0%) | 2(66.7%) | 2(66.7%) | 2(66.7%) |

TS: Total sample; Pat: Pattern; I: Intermediate; R: Resistant; S: Sensitive; AK: Amikacin; AMX: Ampicillin; CN: Cephalexin; CAZ: Ceftazidime; CRO: Ceftriaxone; CIP: Ciprofloxacin; COT: Co-Trimoxazole; GM: Gentamycin; NIT: Nitrofurantoin; NA: Natildic Acid; TE: Tetracycline; E: Chloramphenicol

Table 4: Antibiotic susceptibility pattern of the six most frequent bacterial isolates.

| Bacterial Isolate | TS | Pat | Antimicrobial Agents, n (%) |
|-------------------|----|-----|-----------------------------|
|                   |    |     | E  | RA | VA | CC | OX | P |
| S. aureus         | 3  |     | 1(33.3%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) |
|                   |    |     | R | 0(0%) | 0(0%) | 2(66.7%) | 0(0%) | 0(0%) | 2(66.7%) |
|                   |    |     | S | 2(66.7%) | 3(100%) | 1(33.3%) | 0(0%) | 1(33.3%) | 0(0%) |

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The study results showed a lower rate of an overall growth of bacteria which is 37.4 percent (n=149). Similar cross-sectional study that was done in Iran, from July 2006 to June 2007, also documented a growth for *Klebsiella spp.* (22.4%), *Staphylococcus aureus* (12%), *Citrobacter spp.* (8.8%), and *Escherichia coli* (8.2%), being the most frequent microorganisms [16]. The similarity and differences of the growth rate can be due to difference in the use of equipment, material and setting services related to culture and sensitivity test preparations and supply management. Like many other studies this study also documented *E.coli* isolates as the most prevalent 32.2 percent (n=48) and it was observed to be highly sensitive to chloramphenicol, Gentamycin and Ceftriaxone with a sensitivity rate of 72.9 percent,70 percent and 56.3 percent, respectively; but resistant to Ampicillin and Cephalaxin in 87.5 percent and 72.9 percent. This resistance specifically to Ampicillin, tetracycline, Co - trimoxazole and amoxicillin can be mainly related to the self-administration of common antibiotic drugs because of easy access as routing drugs for therapeutic interventions and as commonly used without prescription even for animals. Antibiotic use continued to be higher in high income countries the agricultural activities in the community have contributed to selection pressure that has sustained resistant strains, forcing to too costly and broad-spectrum antibiotics use.

In developing low-income and middle-income countries (LMICs) antibiotic use is increasing with rising incomes, high rates of hospitalization, and high prevalence of hospital infections [17]. A Retrospective study in Lebanese hospitals also documented a sensitivity of 97.2 percent and 71.7 percent, for Amikacin and Gentamycin, respectively [18]. A study that was conducted in Ethiopia by Dessie [19], for susceptibility test of isolates from surgical site infection documented a similar sensitivity test of 75 percent for Chloramphenicol and resistance for Ampicillin, and Tetracycline in 95.8 and 83.3 percent, respectively [19]. A similar study in Kenya documented a resistance to Ampicillin and Co-trimoxazole of 60 percent, each [20]. Another study in Ethiopia also documented a resistance to Ampicillin (80%) and sensitivity to Gentamycin (66%), Ceftriaxone (53.1%), and Chloramphenicol (51%) [21]. *Klebsiella spp.* also found to be sensitive to Chloramphenicol where as in other studies it was documented as highly sensitive to Amikacin [16]. *Citrobacter spp.* were also found to be resistant to Ampicillin and Cephalaxin in 100 percent and 88.9 percent, respectively; whereas it was found to be sensitive to Amikacin and Ceftriaxone in 61.1 percent, each; and Ciprofloxacin, Gentamycin and Chloramphenicol 55.6 percent, each. The study results showed *Pseudomonas spp.* to be sensitive to Amikacin (72.7%) which is similar to the study of Iran that documented 54 percent sensitivity of Amikacin and 66.6 percent resistance to Gentamycin [16,22]. *Salmonella spp.* were found to be resistant to Ampicillin (100%) and ceftriaxone (66.7%), and sensitive to Amikacin and Ciprofloxacin, ((66.7%), each). Similar resistance pattern for Ampicillin (100%) was also observed in a study conducted in Kenya [20]. A study in Ethiopia also documented to Amikacin and Ciprofloxacin, ((66.7%), each). Similar resistance pattern for Ampicillin (100%) was also observed in a study conducted in Kenya [20]. This similarity of the increase in the pattern of the antimicrobial resistance in the low income Eastern African countries and Eritrea could be attributed to similar climate change, societal belief and culture, access to antimicrobial drugs and achievement to modern medicine [6,17]. Sengupta et al. [23], in their review article documented that even human intervention is a contributing factor for the spread of antimicrobial resistance [23]. The most commonly used medication during the absence of Ampicillin, Gentamycin, Tetracycline and Amoxicillin is Ciprofloxacin.

Currently Ciprofloxacin is started to be sold at drug shops without prescription therefore the development of resistance against this drug is evident. A study in Ethiopia also documented a sensitivity to Ciprofloxacin (100%), and Amikacin (89.5%). However, the study documented the highest resistance for Ampicillin (100%) which is similar to the above studies [24]. *Staphylococcus aureus* also found to be sensitive to Rifampicin in 100 percent and to Co - trimoxazole and Chloramphenicol in (66.7%) of isolates each; whereas resistance was observed for Nitrofurantoin 100 percent, and Gentamycin, Vancomycin, Oxacillin and Penicillin ((66.7%), each). A study conducted by Desse et al. [19], also documented a sensitivity rate for Gentamycin and Ciprofloxacin (184.2%), each, and Tetracycline and Erythromycin in ((78.9%), each) [19]. But a higher resistance rate for penicillin (66.7%) was observed which is similar to the study conducted in Ethiopia and Sudan that documented 94.7 percent and 91.3 percent, respectively [19,25]. In this study high antimicrobial resistance could be attributed to inappropriate specimen selection, collection and preparation, improper laboratory and clinical settings preparation, failure to follow infection prevention control guidelines, malpractices that are related to antibiotic misuse which promote antimicrobial drug resistance [26]. Levy [2], in his paper that entitled “The Challenge of Antibiotic Resistance”, concluded that bacteria insensitivity to an antibiotic appears in a person or in a community mainly due to two forces that include prevalence of resistance genes and the extent of antibiotic use [2].

Therefore, this study has similarity and differences to different studies on antimicrobial resistance that have been done in
many, all the above studies revealed a different resistance and susceptibility pattern of antimicrobial drugs that can be due to increased mobility and globalization that are reducing the time needed for antibiotic-resistant microorganisms to spread simply throughout the world [27-29]. The core action that helps for fighting the spread of antimicrobial resistance is an improved and appropriate use of antimicrobials.

Conclusion and Recommendation

In general the study results revealed that the isolates were sensitive to Chloramphenicol Amikacin, Ciprofloxacain, Ceftriaxone and Gentamycin; whereas they were resistant to Cephalexlin, Ampicillin, Cefazidime, Nitrofurantoin, Co-trimoxazole, Nalidixic Acid and Tetracycline. This resistance is mainly due to antibiotic misuse that includes people's use of drugs for viral infections buying from pharmacies and drug shops without prescription as self-administered drugs for the treatment of health problems that does not require, like colds and flu; and as growth promoters in animals and fish [30].

Therefore, this study will help for policy makers and specifically to the drug administration regulatory body to develop policies regarding the rational drug use and proper classification of drugs based on the level of health facility and human resources deployment which is mainly related to professional hierarchy. The study recommends that the practice of rational drug use mainly for the prescription drugs that include antibiotics to be permissible and continuous surveillance for an antimicrobial drug sensitivity test to be done at different localities, in order to administer drug that is appropriate for treating disease and reducing the emergence of new resistant bacteria and minimizing the spread of resistant microbial isolates.

Competing Interests

All authors of the study declare that they have no conflict of interests associated with the publication of this paper.

Authors’ Contribution

Yemane Seyoum was involved in the designing and proposal writing, interpretation of the study findings, writing and reviewing of the report and final paper preparation. Elias Teages Adgoy was involved, in the designing and proposal writing, in data collection, data analysis and interpretation of the study findings, writing and reviewing of the report and final paper preparation. Kidane Siele was involved in data collection, in the designing and proposal writing, interpretation of the study findings, writing and reviewing of the report and final paper preparation. MohammedElfath was involved in designing and proposal writing of the study, report reviewing and final paper preparation. Mohammed Elfath was involved in designing and proposal writing of the study, report reviewing and final paper preparation. Mohammed Elfath was involved in designing and proposal writing of the study, report reviewing and final paper preparation.

Nesterab Gebreleul was involved in data collection.

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