Prevalence of Multiple Antibiotic Resistant Bacteria in Selected Libraries of University of Ibadan, Nigeria

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Abstract: The health and wellbeing of the public are affected by the physical, chemical and biological properties of their indoor environments. There are many multiple drug resistant pathogenic microorganisms of public health significance found in indoor air and on indoor surfaces which are inadvertently introduced on and into the body through contact. Antibiotic sensitivity of 40 identified bacteria isolated from indoor air and book surfaces in selected libraries in University of Ibadan to different broad spectrum commonly antibiotics was carried out using the disc diffusion method. The settle plate method was used to collect indoor air samples while book surfaces were swabbed using sterile swabs to collect microbial contaminations. Bacteria isolated were species of *Bacillus, Staphylococcus*, *Proteus, Micrococcus*, *Yersenia*, *Erwinia*, *Klebsiella Serratia, Pseudomonas* and *Providencia*. *Bacillus* spp. had the highest occurrence of 27.5% followed by *Staphylococcus* sp. (22.5%), *Erwinia* and *Providencia* spp. had the lowest occurrence of 2.5% each. Resistance of these organisms to the test antibiotics ranged between 17.5% for ciprofloxacin to 75% for tetracycline. Irrespective of species, 46.25% of the isolates were resistant to all the antibiotics while 42.5% were susceptible to them all. Also, 100% were resistant to at least one or more antibiotics while 82.5% were multiple drugs resistant. Three (7.5%) of the Gram positive bacteria isolated from these libraries (*Staphylococcus aureus, Bacillus subtilis* and *Staphylococcus cohnii*) showed 100% resistance to all the tested antibiotics. Results of this study shows that library environments (indoor air and books), can serve as environmental reservoirs of multiple antibiotic resistant bacteria capable of being transferred to other environments through contact with any of these media. Regular disinfection of library environment and proper hand washing with soap free of antimicrobial agents prior to and after handling library materials were recommended to effectively prevent a pathogen's path of transmission from person to person.
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Introduction

Airborne particles like those originating from living microorganisms such as bacteria, fungi, and viruses generally found in most indoor air and surfaces like books, desks, computer keyboards and pads could be of deleterious effect to man as some may be potential infectious agent. Their components have negative effects especially on the health of immunocompromised people (Bhatia, 2011). The risk of airborne infections especially in enclosed environments like the libraries where aerosols can easily build up to infectious level is particularly high (Bhatia, 2011). Studies have shown the increasing evidence of the spread of disease via the aerial route (Begg, 2003). Some of these microorganisms are resistant to most of the available antibiotics for man's use. Singh *et al*. (2011) previously isolated bacteria from books in hospital library that showed resistance to antibiotics. Consequently, the presence of bio-aerosols, and pathogenic microorganisms in public places like the university libraries where students, lecturers, visitors and library staff have contact with each other for several hours through library materials needs to be monitored and controlled to prevent transmission which occurs when microbial pathogens are released from an infected patient to vulnerable individuals through activities such as coughing, sneezing and talking (Durmaz *et al.,* 2005) and ultimately prevent multidrug resistance among pathogens. The development of resistance to antimicrobials has become a serious problem worldwide threatening the ability to treat infections in humans and animals (Adelowo *et al.,* 2008). This has been attributed to the high levels of antimicrobial use in the clinical settings as well as their over-use in agriculture (Martinez and Baquero, 2002; Wassmer *et al*., 2006). According to Gilbert and McBain (2003); Wassmer *et al*. (2006) improper use of antimicrobial agents such as penicillin and erythromycin in the treatment of infections has contributed to the development of antibiotic resistance in pathogenic strains. Widespread inappropriate prescription of antimicrobials, as well as non- adherence to prescription instructions contribute to antibiotic resistance (Wassmer *et al*., 2006). Chigbu and Ezeronye, (2003) and Torimiro *et al*. (2005), stated that there is uncontrolled availability of antibiotics and other drugs in several developing countries including

Nigeria. This has resulted to transmission of antibiotic resistant genes among pathogens. This transmission among pathogenic bacteria has recently become an area of interest in scientific research, because of its close relation to the occurrence and severity of infection affecting human health (Shanks and Peteroy-Kelly, 2009). The knowledge of multiple drug resistant bacteria and the available antimicrobial agents among professionals is necessary to reduce levels of resistance through correct use of antimicrobials and thus prevent unnecessary exposure of bacteria to antimicrobial agents that might provide them a suitable chance to develop and transmit resistant genes by various means. The development and spread of antimicrobial resistant microorganisms is of great importance to public health and when these microorganisms become resistant to antimicrobial agents, they play an important role in the development and persistence of disease (Shanks and Peteroy-Kelly, 2009). The development of resistance among bacteria could be a threat to public health especially in the case of immune-compromised individuals, whose only defense against pathogens is provided by antimicrobials (Shanks and Peteroy-Kelly, 2009).

Closed settings like libraries in University of Ibadan can provide opportunities for the transmission of multiple antibiotics resistant bacteria between the immune-compromised and normal individuals and thereby making the normal individuals to easily become susceptible to opportunistic infection. Therefore informing the public (library staff and other library users) of how antibiotic resistant bacteria are transmitted from person to person and consequently their persistency and failure of therapeutic agents for treating various infections, is vital to lowering the amount of antimicrobial resistance observed in the environment.

Previous studies have shown that formite like soft toys and magazines in the waiting rooms of general practice surgeries are often contaminated with potential bacterial pathogens including coliforms, *Clostridium perfringens*, and *Staphylococcus* (Merriman *et al.,* 2002; Davies *et al.,* 2000; McKay and Gillespie, 2000; Hughes *et al.,* 1986), for which reason, the question was raised as to whether books and magazines were similarly contaminated by pathogenic bacteria and should these materials be removed from hospital waiting rooms (Charnock, 2005; Merriman *et al.,* 2002). Thus the present study not only analyzed the possibility of pathogenic bacteria on books in university of Ibadan libraries and in the indoor air of these libraries but also considered the likelihood of multidrug resistance among the bacteria in order to assess whether these materials are potential environmental reservoir of pathogens and multiple drug resistant bacteria.

Materials And Method

Study Areas

The samples were collected from four different libraries in University of Ibadan, Ibadan, Nigeria. The libraries included; Kenneth Dike library which is the central hub of the library system in the university which was established in the inception of the University in 1948. According to the library guide, (2014), the library is one of the largest in Black Africa South of Sahara housing over-half a million volumes of books and about 6000 periodical tittles (current and non- current). It operates a library system that comprises of 28 Faculty/Departmental Libraries, among which are Microbiology library, Zoology library, and History library where the samples were collected. The library received over 10000 users from the university, excluding users from other universities in the year 2014 when this research was conducted; and three departmental libraries. The research work was conducted between September 2014 and January 2015.

Sampling

A total of sixty samples (comprising of 20 indoor air samples and 40 samples from book surfaces) were collected and analysed. The air samples were collected utilizing the passive air sampling technique, without controlling any indoor environmental condition. The samples were collected by exposing appropriately labeled 9.0cm in diameter Petri dishes containing; Nutrient agar, Mannitol Salt Agar, MacConkey Agar, Samonella Shigella Agar, Eosin Methylene Blue agar and Blood agar within designated areas in the libraries and the microbial air quality was evaluated based on the count of the microbial fallout on to Petri dishes left open to the air according to the 1/1/1 scheme (for 1h, 1m from the floor approximated to human breathing zone, and at least 1m away from walls or any obstacle). After collection, the samples were transported to Microbiology laboratory, University of Ibadan and were incubated at 37° C for 24 - 48 hours.

A total of 40 samples were randomly collected by aseptically swabbing entire book covers and a few pages of the books on the shelves and those being used by students to collect the bacterial contamination using sterile swab. Thereafter, the swab was returned to their tubes and the capped firmly. Collected samples were transported quickly to the microbiology laboratory, university of Ibadan. Exactly 5ml of tryptone soya broth was added to each swab sample for enrichment and were left for a recovery period of 30 minutes at 37ºC. Appropriate dilutions of each sample was inoculated in duplicate onto plates of Nutrient agar, the Mannitol Salt Agar, MacConkey Agar, Samonella Shigella Agar, Eosin Methylene Blue agar and Blood agar. The plates were allowed to stand at room temperature for a recovery period of 1 hour before

being incubated at 37°C for 24 to 48 hours. Both indoor air samples and those from book surfaces collected were collected between 1:00 p.m. and 2:00 p.m. daily since human activity increase at this time in the selected libraries.

Identification of the Isolates

After incubation, the isolates obtained were initially characterized by colony morphology, hemolysis on blood agar, microscopic appearance after Gram staining, growth and characteristic on selective and deferential media. A sub culture of each isolate was then made on appropriate culture media and further identification was done using standard biochemical tests such like catalase, urease, oxidase, hydrogen sulphide test, motility (SIM), coagulase, indole production, citrate utilization, methyl-red and Voges-Proskauer, gelatin hydrolysis, fermentation different sugars including; glucose, lactose, galactose, sucrose, xylose, arabinose, maltose, and mannitol.

Antibiotic Susceptibility test

All the isolates were subjected to antibiotics susceptibility test using modified Kirby-Bauer agar disk diffusion method (Bauer *et al*. 1966) on Mueller-Hinton agar. The isolates where tested against six commonly used antibiotics namely Cefpodoxime (CPD 10µg), Ciprofloxacin (CIP 5µg), Tetracycline (TET 30µg) and Sulphamethoxazole Trimethoprim (SXT 25µg), Ceftazidime (CAZ 25µg), and Chloramphenicol (C 30µg) (Oxoid, Basingstoke, England) and 0.5 McFarland standard suspension was used for standardization of bacterial suspensions. The diameter of the zone of inhibition of 24 hour growth of each isolate was carefully measured and compared to the standard table for antibiotic concentration. The isolates were reported as susceptible, intermediate or resistant according to interpretative chart of complete growth inhibition zone diameter sizes for bacteria according to the Clinical and Laboratory Standards Institute (2011). Data obtained were used to construct the phenotypic pattern of antibiotic resistance for respective organism.

Results

After the removal of isolates of the same species from the same sample, total of 40 bacterial isolates were therefore screened and identified from both samples collected from book surfaces and indoor air samples are summarized on **Table 1** below. Gram positive bacteria were 24(60%) while 16 (40%) represented the Gram negative. *Bacillus* spp. (27.5%) was the predominant isolates followed by *Staphylococcus* spp. (22.5%). *Erwina* and *Providencia* spp. (2.5% each) were the least occurring bacteria in the study. The species/genera of bacteria isolated includes; *Bacillus subtilis, B. cereus, B. megaterium, B. pumilus, Staphylococcus aureus, S. arlattae, S. chonii, S. haemolyticus S. muscae, Proteus mirabilis, Micrococcus luteus, M. carouselicus, Yersinia enterolitica, Erwinia mallotivora, Klebsiella pneumoniae, Serratia marcenscens, Pseudomonas aeruginosa and Providencia sp.*

The result of different species/genera of bacteria (**Table 2**) isolated from the 40 samples randomly collected from books by swabbing indicates that Kenneth Dike library had the highest number of bacterial species/genera (56.7%), a relatively high number (23.3%) was observed in History library while Microbiology and Zoology libraries had the least number of (10%) each. Also, the most abundant bacterial species were *Bacillus* (30%) among which *Bacillus subtilis* was the highest, occurring in all the libraries except in Zoology library while *Erwina* and *Providencia* spp. were the lowest (3.3%) each occurring bacteria.

Different species/genera of bacteria were isolated from the 20 indoor air samples collected from the designated libraries of university of Ibadan are summarized on **Table 3.** Kenneth Dike library again had the highest number (60%) of different bacterial species. This was followed by History library with 20% of the total isolates. *Klebsiella pneumonia* (Gram negative bacteria) were the predominant (30%) among the identified bacteria from the indoor air samples.

Ciprofloxacin (**Figure 1**) was the most effective antibiotics in this study, as only 7 (17.5%) of the isolate were resistance to it. Generally, most of the isolates were resistant to tetracycline 30 (75%) followed by Ceftazidime 20 (50%), Cefpodoxime 18 (45%), while Chloramphenicol was 17 (42.5%). Only 6 (15%) of the isolate were susceptible to tetracycline, 25 (62.5%) to Ciprofloxacin while 21 (52.5%) were susceptible to Sulphamethoxazole/Trimethoprim.

Table 1. Relative Proportion of Species/Genera of Bacteria Isolated From four selected Libraries in University of Ibadan.

Isolates	Number of isolates	Percentage
Gram-positive		
Bacillus	11	27.5
Staphylococcus	9	22.5
Micrococcus	4	10
Gram-negative		
Proteus	4	10
Pseudomonas	2	5
Yersinia	3	7.5
Serretia	2	5
Erwinia	1	2.5
Providencia	1	2.5
Klebsiella	3	7.5
Total	**40**	**100**

Generally evaluating the 40 isolates irrespective of their species showed that 46.25% were resistant to all the antibiotics while 42.5% were susceptible to them all. Twenty two phenotypic patterns of drug resistance were observed among the bacteria isolated from selected libraries in University of Ibadan (**Table 4).** All of patterns were multiple antibiotic resistance patterns with the least being resistance to one drugs by grouping. Of the 40 isolates, 100% were resistant to at least one or more antibiotics while 82.5% were resistant to two or more antibiotics. Three (7.5%) of the Gram positive bacteria isolated from these libraries (*Staphylococcus aureus, Bacillus subtilis* and *Staphylococcus cohnii*) showed 100% resistance to all the antibiotics used in this research.

Table 2. Bacterial isolates from book surfaces in the selected University of Ibadan libraries

Libraries	Bacteria	Percentage
Kenneth Dike Library	*Staphylococcus aureus* (2) *Micrococcus luteus* *Yersinia* sp. *Erwina mallotivora* *Yersinia* sp. *Bacillus cereus* *Staphylococcus cohnii* *Bacillus subtilis* (2) *Proteus mirabilis* *Bacillus* sp. (2) *Bacillus megaterium* *Micrococcus carouselicus* *Staphylococcus arlettae* *Yersinia enterolitica*	56.7
History Library	*Proteus* sp. *Proteus mirabilis* (2) *Pseudomonas aeruginosa* *Staphylococcus* sp. *Bacillus subtilis* *Staphylococcus arlettae*	23.3
Microbiology library	*Bacillus subtilis* *Bacillus pumilus* *Staphylococcus muscae*	10
Zoology library	*Pseudomonas aeruginosa* *Providencia* sp. *Serratia marcescens*	10
	Total	**100**

Table 3. Bacteria isolates from indoor air samples in the selected libraries in University of Ibadan

Libraries	Bacteria	Percentage
Kenneth Dike Library	*Micrococcus* sp. *Klebsiella pneumonia* (2) *Bacillus cereus* *Micrococcus luteus* *Staphylococcus haemolyticus*	60
History Library	*Staphylococcus arlettae* *Serratia marcescens*	20
Microbiology Library	*Klebsiella pneumoniae*	10
Zoology Library	*Bacillus subtilis*	10
	Total	**100**

Table 4. Phenotypic patterns of antibiotic resistance among isolates from University of Ibadan Libraries

Resistant pattern	**Bacteria**	**Nos. of Isolates**
CAZ	*Micrococcus luteus*	1
	Erwinia mallotivora	1
CIP	*Staphylococcus arlettae*	1
	Micrococcus carouselicus	1
TET	*Yersinia* sp.	1
	Yersinia enterolitica	1
	Micrococcus luteus	1
C, CAZ	*Pseudomonas aeruginosa*	1
C, TET	*Bacillus subtilis*	2
CPD, CAZ	*Yersinia* sp.	1
	Bacillus subtilis	1
CAZ, TET	*Bacillus Megaterium*	1
	Bacillus cereus	1
	Staphylococcus haemolyticus	1
	Staphylococcus muscae	1
CDP, TET	*Micrococcus* sp.	1
SXT, CAZ	*Klebsiella pneumonia*	1
SXT, TET	*Klebsiella pneumonia*	1
C, CAZ, TET	*Bacillus cereus*	1
C, SXT, CPD	*Serratia marcescens*	1
	Klebsiella pneumonia	1
C, CPD, TET	*Klebsiella pneumonia*	1
	Providencia sp.	1
	Staphylococcus arlettae	1
SXT, CIP, TET	*Proteus* sp.	1
SXT, CPD, TET	*Staphylococcus arlettae*	1
	Bacillus pumilus	1
	Pseudomonas aeruginosa	1
SXT, CAZ, TET	*Proteus mirabilis*	2
C, CPD, CAZ, TET	*Bacillus* sp.	1
C, CPD, CIP, TET	*Serratia marcescens*	1
C, SXT, CPD, TET	*Proteus mirabilis*	1
C, SXT, CAZ, TET	*Bacillus* sp.	1
	Staphylococcus sp	1
CPD, CIP, CAZ, TET	*Staphylococcus aureus*	1
C, SXT, CPD, CIP, CAZ, TET	*Staphylococcus aureus*	1
	Bacillus subtilis	1
Total	*Staphylococcus cohnii*	1 **40**

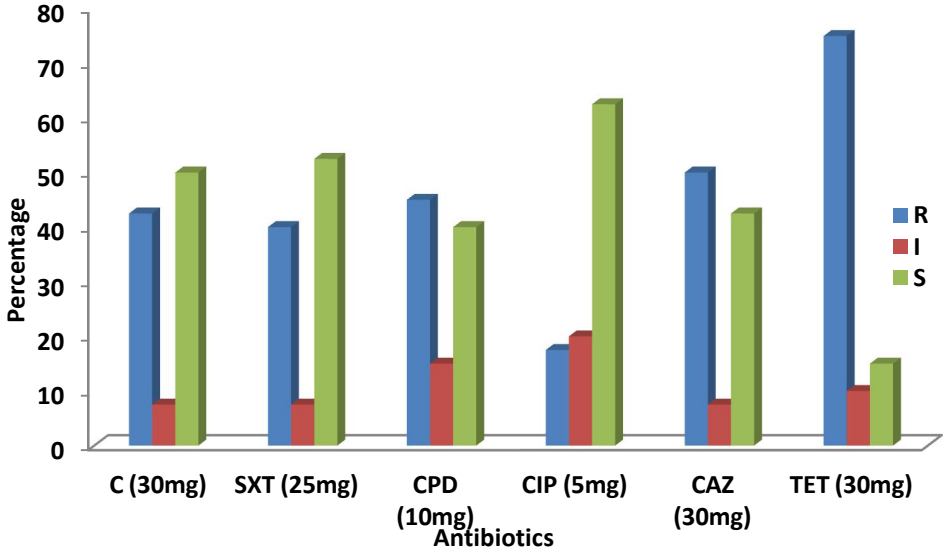

Figure 1. Test antibiotics (µg) and bacterial sensitivities.
Key: C=Chloramphenicol; SXT=Trimethoprim/sulfamethoxazole; CPD=Cefpodoxime; CIP= Ciprofloxacin; CAZ=Ceftazidime; TET=Tetracycline. R=Resistance; I=Intermediate; S= Susceptible.

Discussion

If an organism is resistant to a specific antimicrobial, it implies that the antimicrobial will not be effective against that organism if it causes an illness. In this study, we isolated and identified 40 bacteria species from four different libraries in university of Ibadan and tested them against 6 commonly used broad spectrum antibiotics.

The bacterial species identified (**Table 1**) most of which were of public health importance and include species of *Bacillus*, *Stahpylococcus*, *Proteus*, *Klebsiella pneumoniae*, *Serratia marscences, Pseudomonas aeruginosa*, *Yersinia*, and *Micrococcus*, *Providencia, Erwinia*. The preponderance of Gram-positive bacteria over the Gram-negative bacteria substantiates the result of Fox *et al*. (2005) who stated that when a building is occupied, the concentrations of Gram-positive bacteria may be elevated and decreases in Gram-negative bacteria population may occur. Also, the isolation of Gram-positive cocci belonging to saprophytic microflora like *Staphylococcus* and *Micrococcus* species (implicated to be associated with human skin and mucosa) in these libraries, suggests that the bacterial contamination suspended in the indoor air and book surfaces were as a result of the frequent presence of human just like was discovered in previous study (Hayleeyesus and Manaye, 2014). Of all the libraries sampled, Kenneth Dike was observed to have the highest number of bacterial species in both indoor air samples and samples collected from book surfaces. This was probably because of the relative sizes (the largest in Nigeria), number of people always present and the numerous commercial activities therein.

There were generally multiple resistances among the organisms isolated from the selected libraries to the tested drugs. The high resistance noticed in our study is in corroboration with previous study of Adelowo *et al*. (2009), resistance ranged from 17.5% for ciprofloxacin to 75% for tetracycline. The high number of antibiotic resistance by most of the bacterial isolates to tetracycline observed in our study is in consonant with previous study by Adelowo *et al*. (2009) where they also observed tetracycline to be the least effective among the antibiotics used in their study. Half (50%) of the isolates were resistant to ceftazidime. Ciprofloxacin was typically the most effective drug against the isolates as majority (62.5%) of the organisms was susceptible to the drug. This finding perfectly agrees with the previous work of Thomson (1999) who stated that ciprofloxacin is usually the last resort for the treatment of difficult infections because of its potency in treatment where other antibiotics have failed to treat. Judging from our findings, it is safe to imply that ciprofloxacin could be the drug of choice in treating illnesses caused by these environmental microorganisms.

The multiple antibiotic resistance of these bacteria (**Figure 1**) calls for an immediate attention from all the concern agencies as all these bacteria were isolated from library environment where there is high possibility of transmission of these pathogens among students, staff and other library users and consequently may reach the larger society. With this increase in

development and spread of multiple antimicrobial resistances among microorganisms in the environment, treatment of illness becomes difficult to achieve with a single antimicrobial agent (Thomson, 1999; Martinez and Baquero, 2002; Wassmer *et al*., 2006).

The resistant patterns of isolates revealed varying degrees of resistance to the antibiotics tested (Table 4). Our study demonstrates an evidence of rapid development and spread of multiple drug resistance where about 100% and 82.5% of the isolate were resistant to at least one and two or more respectively. This observation was comparatively higher than those of Leta *et al*. (2009) where they recorded 86.9% resistance to 1 or more antibiotics and 73.8% resistant to 2 or more antibiotics. These levels of resistance seen among bacterial isolates in this work were generally the same as those of clinical isolates in recent studies in Nigeria (Adelowo *et al*, 2008, Chigbu and Ezeronye 2003) from a completely different point of isolation.

Some of the microorganisms like *Staphylococcus aureus, Staphylococcus cohnii* and *Bacillus subtilis* (**Table 4**) isolated from these libraries which were resistance to a number of the drug used in our work was not surprising as the same organisms have been observed also in previous similar studies (Suller and Russell, 2000; Martinez and Baquero, 2002). According to Martin *et al.* (2005) more than 70% of the bacteria that causes nosocomia infections are resistant to at least one of the drugs most commonly used to treat the infections they cause, in agreement with this, the findings of the present study indicated that 88.9% of *Staphylococcus* species (known to be one of the leading cause of nosoconia) showed resistance to two or more of the tested antibiotics. The isolation of multiple antibiotic resistant *Staphylococcus* species from these environments (where most are normal flora of the skin, nose and mouth) is worrisome considering the location and the population of people that might be involve in contact with each other. Except for one particular strain of *Bacillus subtilis,* which showed 100% resistance to the tested drugs, others were significantly sensitive to the antibiotics used.

Most of the isolates were more susceptible to the newer class of antimicrobials like Ciprofloxacin, Trimethoprim/Sulfamethoxazole, than the older generations of antimicrobials. Therefore from this study, if a choice of antibiotics is to be made for the treatment of infection caused by any of the 40 organisms, it will be in the order: Ciprofloxacin > Trimethoprim/Sulfamethoxazole > Chloramphenicol > Ceftazidime > Cefpodoxime > Tetracycline.

In this study, the isolation of loads of antibiotic resistant bacteria from the selected libraries (non-clinical environments) justifies the previous work of Gaza *et al,* (2008) who stated that it has long been suspected that the environment constitutes a reservoir of novel antibiotic resistance genes, although its significance has been overlooked in favour of evolution of resistance within the clinical environment.

Conclusion

This study revealed a worryingly high level of antimicrobial resistance among bacteria isolated from selected libraries of university of Ibadan considering the fact that 100% of the isolates were resistant to one or more of the drugs while 82.5% were resistant to at least two of the antimicrobials. Prominently among these isolates are the Staphylococcal species associated with the skin, nose and mouth; *Bacillus* species and *Klebsiella* species. This indicates how unsafe these environments are because of the possible risk of spreading of these pathogens and resistant genes among the library users and consequently the public.

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