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Clinicobacteriological study of Urinary tract infection in pregnant women.

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Abstract:
Introduction: *Untreated asymptomatic bacteriuria in pregnancy leads to number of complications such as acute and chronic pyelonephritis, toxemia, anaemia, hypertension, prematurity, intrauterine growth retardation and increased perinatal mortality. These complications can be prevented by early detection and treatment of asymptomatic bacteriuria in pregnant women.*
Material and methods: *A Total of 460 urine samples were collected for study. Both macroscopic and microscopic examination were done. Plating of urine sample was done on Blood agar, Mac conkey agar by standard loop technique. Colony count was measured for interpreting significant bacteriuria. Organism was identified with standard biochemicals. Antibiotic sensitivity was performed as per CLSI 2015 guideline.*
Observation: *Incidence of significant bacteriuria in study group was 10.21%. Incidence in control group was found to be 4%. Significant bacteriuria was found in 8.16% primigravida cases and 11.74% multigravida cases. Percentage of significant bacteriuria was higher in third trimester i. e.11.8%.*
In symptomatic cases most common symptom was burning micturition (47.05%) followed by increased frequency (35.29%). Escherichia coli was observed as a predominant organism in a 55.31% cases. Organisms were found resistant to many of commonly used antimicrobials.
Conclusion: *Most cases were of UTI were asymptomatic. Also isolated organism were resistant to many routinely used antimicrobials. As these cases can lead to further complications, pregnant women should undergo screening of UTI during pregnancy.*
Keywords: *Asymptomatic bacteriuria, Significant bacteriuria ,UTI in pregnancy*

I. Introduction

Urinary tract infection is one of the most common reason for the people to seek medical consultation. Urinary tract infection affects all age group and both gender[1]. Urinary tract infection is fourteen times more common in females than males[2], reason could be short urethra, easy contamination of urinary tract with fecal flora and various other reasons such as obstruction either mechanical or functional. Obstruction leads to stasis of urine which forms good culture medium for the organism[3].

Urinary tract infection is most common bacterial infection in women. Furthermore, urinary tract infection is second most common medical complication of pregnancy (second only to Anaemia)[2]. Reason may be mechanical factor such as compression of urethra due to enlarging uterus, hormonal factors that leads to decrease ureteric motility and bladder tone and promoting the adhesion of germs to the urothelium, chemical factor such as alkalination of urine and physiological glycosuria, and increase in bacteria in the vulvoperianal region during pregnancy[4].

The urinary tract infection have three typical presentations, Asymptomatic bacteriuria (ASB), Acute cystitis and Pyelonephritis[5].

Asymptomatic bacteriuria, is defined as the presence of greater than or equal to 10^5 colony forming units (CFU) of bacteria per ml of the same single species in two consecutive cultures of clean-voided specimens of midstream urine from an individual without symptoms of a urinary tract infection (UTI). The reason to repeat the culture is to discriminate between true bacteriuria and contamination[6,7]

Significant bacteriuria, is defined as the presence of at least 10^5 CFU/ml bacterial species in urine[8]. Significant bacteriuria may exist in asymptomatic patients and there is subsequent increased risk of developing cystitis and pyelonephritis in untreated patients with ASB[5].

Around 4-10% of all pregnancy manifest with urinary tract infection and untreated patient leads to development of symptomatic cystitis in 30% and pyelonephritis in up to 50% of patient[5]. Untreated ASB leads to number of complications such as acute and chronic pyelonephritis, toxemia, anaemia, hypertension, prematurity, intrauterine growth retardation and increased perinatal mortality [5,9]. The progression of the asymptomatic bacteriuria to symptomatic Urinary Tract Infection in the latter life can be prevented by early detection and treatment of asymptomatic bacteriuria in pregnant women.

Escherichia coli is the most frequent cause of urinary tract infection, others are *Klebsiella* species, *Pseudomonas* species, *Proteus* species, *Enterobacter, Staphylococcus aureus, Enterococcus faecalis* and some times coagulase negative *Staphylococci*[10,11]

The American Academy of Pediatrics recommended, screening for asymptomatic bacteriuria "early in pregnancy, as appropriate".[12]

In view of the above, an attempt was made to evaluate the urinary tract infection in pregnancy.

II. Material And Methods

The present study was undertaken in Department of Microbiology, S.R.T.R.Government Medical College and Hospital, Ambajogai, Maharashtra..

A Total of 460 urine samples were collected for study, from women attending antenatal clinic for routine check up, during the study period. Subjects comprised of varying age from 18 to 35 years, varying gravida and all three trimesters were studied. Urine sample from a group of non-pregnant females of matched age group were also included in the study for control purpose. Information regarding age, parity, history of urinary complaints, past history of diabetes and hypertension was documented.

Exclusion criteria: Patients who were receiving any antibiotics.

Collection of urine sample:

Early morning mid-stream urine samples were collected, in a sterile wide mouthed container. Urine specimens were transported to microbiology laboratory and processed within two hours.

Examination of urine:

1- Macroscopic examination:

Urine was observed for altered colour, presence of any turbidity and the findings were recorded.

2- Microscopic examination:

A] Wet mount examination: [13]

About 10 ml of urine was taken into a conical tube and centrifuged at 1500 rpm for 5 minutes. One drop of sediment was examined microscopically by using 40x. Motility of organism, Pus cells, Red cells, various Casts and Crystals were observed. Presence of more than 10 pus cells per high power field was considered as significant.

B] Gram stain:[5]

The smear of uncentrifuged urine was heat fixed and stained by Gram's stain. Presence of at least one organism per field was considered as significant.

3- Plating of urine sample:

Plating of urine sample was done prior to the other tests.

Standard loop technique

Plating of urine was done by Standard loop technique (internal diameter 4mm to deliver 0.01ml of urine) on Blood agar and Mac Conkey's agar, was as follows-

Method:[11]

1) Urine was mixed thoroughly.
2) The sterile calibrated loop was vertically inserted into the urine.
3) The loopful of urine was spread across the all four quadrants of plate.
4) Plates were incubated at 37^{o}C for 24 hours in an incubator.
5) Colonies were counted on each plate.
6) Significant bacteriuria was confirmed by colony count (multiplying 100 to total number of colonies formed).

Preliminary tests including Gram stain, Hanging drop preparation and Oxidase test were done. Fermentation of sugars including Glucose, Mannitol, Lactose and Sucrose. Other biochemical test like Indole test, Methyl-red test, Citrate utilization, Urease production, H_2S production, Catalase test and Coagulase test were done to identify organisms,by using standard reagents, and standard methods as described by Bailey and Scott's.[11]

3) Antibiotic sensitivity test:[14]

The antibiotic susceptibility testing was performed by Kirby-Bauer Disk diffusion method. Readymade antibiotic discs obtained from Hi-Media Pvt. Ltd, Mumbai was used. Interpretation was done as per CLSI 2015 guideline.

III. Observation

The present study comprised of 460 urine samples of pregnant women attending the antenatal clinic and 50 urine samples as a control group. Routine urine analysis including microscopic examination was done. Culture done by using standard loop technique. Antimicrobial sensitivity was done by Kirby-Bauer disc diffusion method.

Table-1: Showing distribution of significant bacteriuria and non significant bacteriuria in pregnant women. (n=460)

Sr. no.	Type of Cases	No. of cases	Percentage
1	Significant bacteriuria	47	10.21%
2	Non-significant bacteriuria	413	89.78%
3	Total	460	100%

Out of 460 urine samples of pregnant women, 47 cases (10.21%) were found to had significant bacteriuria (1,00,000 or more bacteria/ ml of urine) . Two cases from the control group revealed significant bacteriuria, from 50 urine samples, giving an incidence to be 4%

Table-2: Showing distribution of symptomatic and asymptomatic cases. showing significant bacteriuria.

Sr. no.	Type of cases	Total no. of cases	No. of cases with significant bacteriuria	Percentage
1	Symptomatic	17	6	35.29%
2	Asymptomatic	443	41	9.25%
Total		**460**	**47**	
3	Control	50	2	4%

Most of the cases with significant bacteriuria. were from the age group of above 35 years (40%), followed by the age group of 31-35 years (34.48%) and (9.09%) in the age group of 21-25 years.

Table-3: showing distribution of cases with significant bacteriuria as per gravida.

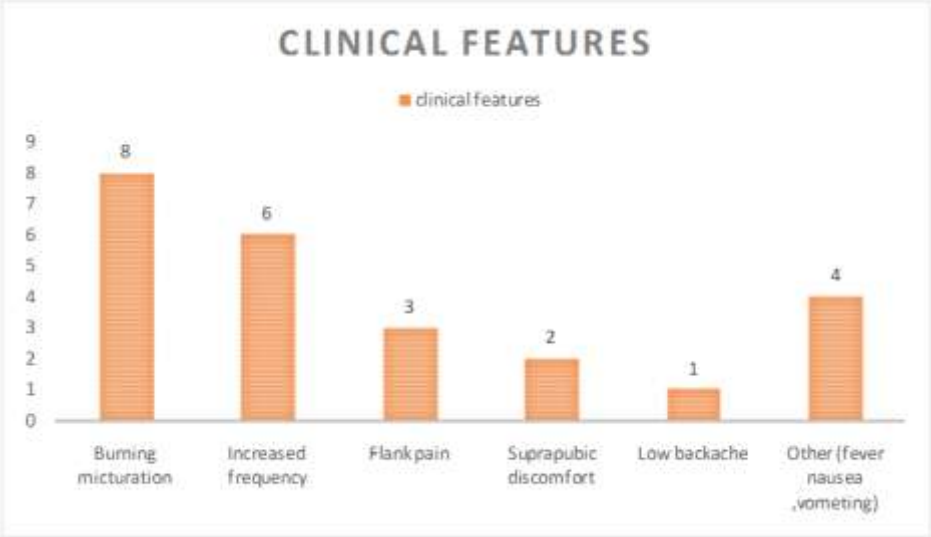
Sr. no.	Gravida	Total no. of cases	No. of cases with significant bacteriuria	Percentage
1	Primi-gravida	196	16	8.16%
2	Multi-gravida	264	31	11.74%
Total		**460**	**47**	

Our study consisted 196 primigravida and 264 multigravida, out of 196 cases of primigravida significant bacteriuria was found in 16 (8.16%) cases and out of 264 cases of multigravida, significant bacteriuria was observed in 31 (11.74%).

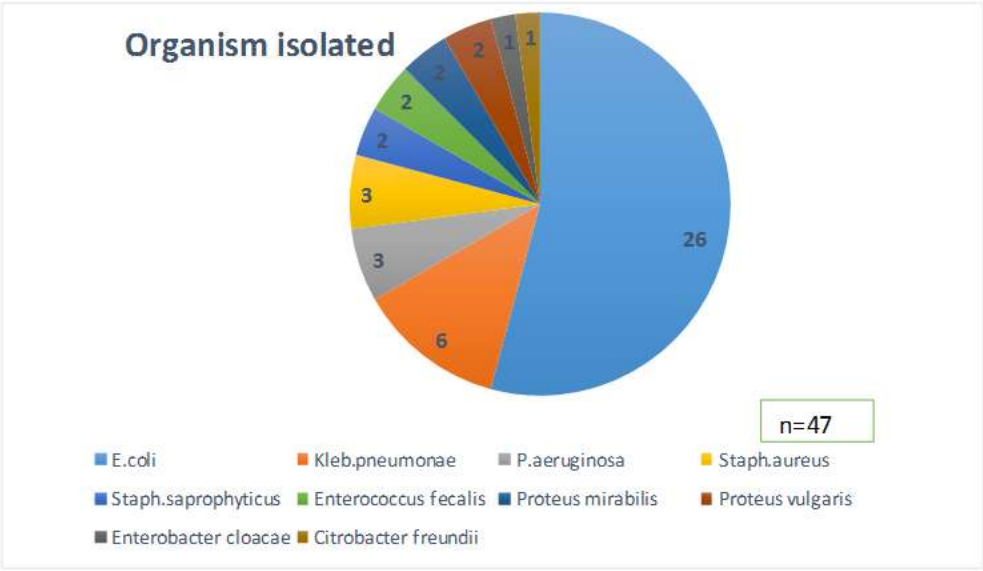
Table-4 : Showing distribution of cases having significant bacteriuria according to duration of pregnancy.

Sr.no.	Duration of pregnancy	Total no. of cases	No. of cases with significant bacteriuria	Percentage
1	First trimester	44	2	(4.54%)
2	Second trimester	145	13	(8.96%)
3	Third trimester	271	32	(11.8%)
Total		**460**	**47**	

Considering months of pregnancy, percentage of significant bacteriuria was higher in third trimester 32(11.8%) cases, as compared to second trimester 13(8.96%) cases and first trimester 2(4.54%) cases.



In symptomatic cases most common symptom was burning micturition (47.05%) followed by increased frequency (35.29%) also symptoms like flank pain (17.64%), suprapubic discomfort (11.76%) and low back pain (5.88%) were also present.



Pie diagram showing number of organism isolated.

Out of 460 cases 47 cases had significant bacteriuria by standard loop technique. *Escherichia coli* was observed as a predominant organism in a 26(55.31%) cases. *Klebsiella pneumonae* was next common isolate, which account for 6(12.76%) of isolation.

The other isolates were, *Pseudomonas aeruginosa* 3(6.38%), *Staphylococcus aureus* 3(6.38%), *Staphylococcus saprophyticus* 2(4.25%), *Enterococcus faecalis* 2(4.25%), *Proteus mirabilis* 2(4.25%), *Proteus vulgaris* 1(2.12%), *Enterobacter cloacae* 1(2.12%) and *Citrobacter freundii* 1(2.12%).

Table 5: Antibiotic sensitivity pattern of urine isolates by disc diffusion method.

Organism	AMP	AMX	AMC	CTX	CXM	FO	NIT	COT
***E.coli* (n=26)**	9 (34.61)	10 (38.46)	12 (46.15%)	17 (65.38%)	10 (38.46%)	22 (84.61%)	23 (88.46%)	16 (61.53%)
***K.pneumonae* n=6**	3 (50%)	3 (50%)	4 (66.6%)	4 (66.6%)	4 (66.6%)	6 (100%)	6 (100%)	3 (50%)
***P.aeruginosa* n=3**	1 (33.3%)	1 (33.3%)	2 (66.6%)	2 (66.6%)	1 (33.3%)	2 (66.6%)	2 (66.6%)	0
***P.mirabilis* n=2**	1 (50%)	1 (50%)	2 (100%)	2 (100%)	1 (50%)	2 (100%)	0	1 (50%)
***P.vulgaris* n=1**	0	0	0	1 (100%)	1 (100%)	1 (100%)	0	1 (100%)

C.freundii **n=1**	0	0	1 (100%)	1 (100%)	0	1 (100%)	1 (100%)	0
E.cloacae **n=1**	0	0	1 (100%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)	0

AMP- Ampicillin, AMX- Amoxicillin, AMC- Amoxicillin/Clavulinic acid, CTX- Cefotaxim, CXM-Cefuroxime, FO- Fosfomycin, NIT- Nitrofurantoin, COT- Co-trimoxazole.

Organism	PEN	CX	AMC	CIP	LZ	NX	NIT	COT
S.aureus **n=3**	1 (33.3%)	1 (66.6%)	2 (66.6%)	3 (100%)	3 (100%)	2 (66.6%)	2 (66.6%)	2 (66.6%)
S.saprophyticus **n=2**	1 (50%)	2 (100%)	2 (100%)	2 (100%)	1 (100%)	2 (100%)	2 (100%)	1 (50%)
E.faecalis **n=2**	1 (50%)	1 (50%)	2 (100%)	2 (100%)	1 (50%)	1 (50%)	1 (50%)	----------

PEN- Penicillin, CX- Cefoxitin, AMC –Amoxycillin –clavulanic acid, CIP- Ciprofloxacin, LZ-Linezolid, NX-Norfloxacin, NIT- Nitrofurantoin, COT- Co-trimoxazole.

IV. Discussion

Urinary tract infection (UTI), is one of the most common reason for the people to seek medical consultation.[1] UTI is most common bacterial infection in women.[2] Pregnancy is one of the common predisposing factor for UTI, which can remain unnoticed due to asymptomatic presentation and can lead to various maternal and foetal complications. Hence, by screening and aggressively treating pregnant women having UTI, it is possible to significantly decrease the various complication during pregnancy due to UTI.[5]

Ever since Kass (1956), drew attention of the medical world to bacteriuria during pregnancy and its effects on the mother and the foetus, the study of bacteriuria during pregnancy received an impetus and since then it has been the subject of excellent studies and reviews.[15,16] The present study was undertaken to enveil bacteriuria during pregnancy, the microorganism responsible and their antibiotic sensitivity. In present study, majority of the cases studied were from low socio-economic conditions and belong to rural area.

The present study was comprised of 460 urine samples of pregnant women, amongst which 47 (10.21%) cases showed significant bacteriuria. The incidence of significant bacteriuria reported in studies done by Shazia parveen et. al[17] in 2011 (7.7%) and Nawaz et. al[18] in 2012 (10.4%) correlates with our study.

The frequency of significant bacteriuria in control group was 4% which was less, as compared to study group 10.21%.

In the present study, 17(3.69%) pregnant women had symptoms suggestive of urinary tract infection, of which 6(35.29%) cases yielded positive for significant bacteriuria.

The study correlated with Deshmukh J and Deshpande A. (1985) [19] reported, 80% culture positive cases of symptomatic significant bacteriuria, where as 38% culture positive cases of symptomatic bacteriuria were reported by Nath et al (1996).[20]

Present study shows, 9.25% cases of significant bacteriuria, in asymptomatic pregnant women. The incidence of asymptomatic bacteriuria in studies done by Kalantar Enayat et. al[1] (2008) was 8.9% , R. J. Girishbabu et. al[21] (2011) was 10%, C. Obirikorang et. al[22] (2012) was 9.5%. which correlate with our study. While, low incidence rates were reported by Kass E. H. (1960) [23] 6%, Nath et. al. (1996)[20] 4% and Perera J. et. al (2012) [24] 3.6%.

The present study shows that, burning micturation was found in 47.05%cases, which was the commonest complaint of UTI, followed by increased frequency in (35.29%) and flank pain (17.64%) cases. R. A. Husain et. al (1994),[3] reported burning micturation was a commonest presentation of UTI, followed by increased frequency. Sushma S. Thakre et. al (2012) [25] also found that, commonest complaint of UTI was burning micturation (55.2%), followed by increased frequency of micturation (51.7%), fever with chills (24.1%), dysuria (17.2%) and loin pain (10.3%).

The present study showed that high incidence of significant bacteriuria noted in multigravida (11.74%) as compared to primigravida (8.16%).The finding was correlated with Roy S.K. et. al (1974),[26] reported 12.2% cases of significant bacteriuria in multigravida women. Nawaz et. al (2012),[18] reported 8.7% cases of significant bacteriuria in primigravida and (11.6%) in multigravida women.

However some workers reported higher incidence of significant bacteriuria in primigravida. Nath et al(1996),[20] reported (11.4%) cases of significant bacteriuria in primigravida and (7.04%) in multigravida women. While, Lavanya S.V. et. al (2002),[27] observed (66.6%) cases of significant bacteriuria in primigravida and (33.4%) in multigravida women.

In the present study, cases studied in the first trimester were 44, in second trimester 145 and in third trimester 271. In the present study, it was observed that, incidence of significant bacteriuria was highest in third trimester (11.8%), followed by second trimester (8.96%) and least in first trimester (4.54%).

Similar findings was observed by Nath et. al (1996),[20] while Girishbabu et. al (2011)[21] observed equal incidence of significant bacteriuria in second and third trimester and Nawaz et. al (2012)[18] although found highest incidence of significant bacteriuria in third trimester, but not much significant differences in incidence of significant bacteriuria in all three trimesters. Increased incidence during third trimester may relate to increased mechanical obstruction due to gravid uterus.

Out of 460 cases 47 cases had significant bacteriuria by standard loop technique. *Escherichia coli* was observed as a predominant organism in a 26 (55.31%) cases. *Klebsiella pneumonae* (6) i.e.12.76% was next common isolate.

The other isolates were, *Pseudomonas aeruginosa* 3(6.38%), *Staphylococcus aureus* 3(6.38%), *Staphylococcus saprophyticus* 2(4.25%), *Enterococcus faecalis* 2(4.25%), *Proteus mirabilis* 2(4.25%), *Proteus vulgaris* 1(2.12%), *Enterobacter cloacae* 1(2.12%) and *Citrobacter freundii* 1(2.12%).

Our study correlates with organism isolated from urine of pregnant women in some important studies as follows

Sr. no	Author	Year	*E.coli*	*K.pneumonae*
1.	Delzell J.E. et. al[5]	2000	80.9%	--
2.	Lavanya S.V. et. al[27]	2002	88%	9.3%
3.	A.B.M.Selimuzzaman et. al[28]	2006	94.83%	1.72%
4.	Gayathree et. al[29]	2010	51.61%	9.67%
5.	Girishbabu et. al[21]	2011	30%	30%
6.	Meher Rizvi[30]	2011	41.9%	21.7%
7.	C.Obirikorang et. al[22]	2012	36.8%	26.3%
8.	Shirish K.P. et. al[31]	2012	53.38%	18.9%
9.	Sushma Thakre et. al[25]	2012	62.06%	6.9%
	Present study	2015	55.31%	12.76%

The next common urine isolates were, *Pseudomonas aeruginosa* 3(6.38%) and *Staph. aureus* 3(6.38%).

Gayathree et. al(2010),[30] found (4.83%) *Pseudomonas aeruginosa* and (9.67%) *Staph. aureus* in urine sample of pregnant women.Girishbabu et. al (2011),[21] reported (7%) *Pseudomonas aeruginosa* and (4%) *Staph. aureus* in urine sample of pregnant women.

Antibiotic sensitivity of urine isolates by disc diffusion method:

In present study, out of 26 isolates of *Escherichia coli*, (88.46%) were sensitive to Nitrofurantoin, (84.61%) were sensitive to Fosfomycin, (65.38%) sensitive to Cefotaxim, (46.15%) were sensitive to Amoxicillin/Clavulinic acid, (61.53%) were sensitive to Cotrimoxazole, (38.46%) were sensitive to Cefuroxime and Amoxicillin and (34.61%) were sensitive to Ampicillin.

Rafique et. al(2002),[32] reported (90.5%) cases of *Escherichia coli* were sensitive to Nitrofurantoin, followed by (62.5%) were sensitive to Cefotaxim, (23.8%) were sensitive to Ampicillin and (21.5%) were sensitive to Cotrimoxazole.

Jean marie et. al(2007),[33] reported (99.3%) of *Escherichia coli* were sensitive to Fosfomycin, (96.5%) were sensitive to Cefotaxim, (89.9%) were sensitive to Nitrofurantoin, (32.5%) were sensitive to Amoxicillin/Clavulinic acid, (31.9%) were sensitive to Cotrimoxazole and (26.9%) were sensitive to Amoxicillin.

Shirish Kumar et. al(2012),[31] reported (80%) isolate of *Escherichia coli* were sensitive to Nitrofurantoin, (76%) were sensitive to Cefuroxime and (72%) were sensitive to Ampicillin.

In present study, out of 6 *Klebsiella pneumonae*, 6(100%) isolates were sensitive to Nitrofurantoin and Fosfomycin, (66.6%) were sensitive to Cefotaxim, Cefuroxime and Amoxicillin/Clavulinic acid, (50%) were sensitive to Ampicillin, Amoxicillin and Cotrimoxazole.

Shirish Kumar et. al(2012),[31] reported (57%) isolates of *Klebsiella* were sensitive to Nitrofurantoin, (78%) were sensitive to Cefuroxime and (61%) were sensitive to Ampicillin.

Kasi Murugan et. al(2012),[34] reported (75%) isolate of *Klebsiella* were sensitive to Nitrofurantoin, (60%) were sensitive to Cefotaxim and only (12.5%) were sensitive to Amoxicillin.

V. Conclusion

Urinary tract infection were observed in 10.21% (47/460) cases of pregnant women. Most cases were asymptomatic, with prevalence rate of asymptomatic bacteriuria in pregnant women were 9.25%. Also isolated organism were resistant to many routinely used antimicrobials. These cases can lead to further complications like, acute and chronic pyelonephritis, toxemia, anaemia, hypertension, prematurity, intrauterine growth

retardation and increased perinatal mortality. The progression of the asymptomatic bacteriuria to symptomatic Urinary Tract Infection in the latter life can be prevented by early detection and treatment of asymptomatic bacteriuria in pregnant women. Hence screening of pregnant women for UTI is necessary.

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