

Distribution of Extend Spectrum Beta Lactmase Enzyme among Escherichia Coli Isolated from Urinary Tract Infection

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ABSTRACT

E. coli is an opportunistic nosocomial pathogen causing a variety of infections including urinary tract infections, pneumonia, septicemia, wound infections, isolated from different clinical specimens that producing extended-spectrum β -lactamase (ESBL) are increasing resist to antibiotics all over the world by distributed between patient with high morbidity and mortality. Our study aimed to carry out a retro spective study on *E. coli* isolates from urine in Erbil hospital from august to February of 2017 and analyze its epidemiology, antibiotics susceptibility pattern, pathogenic potential, and detect frequency of ESBL enzyme among isolates. A total of 399 samples were collected from urine in Rizgary hospital from male and female *E. coli*. Isolated and identified by using microscopical, morphological, biochemical tests and Api and Vitek 2 compact system. Antibiotic susceptibility testing was performed by using Vitek 2 compact system according to the standard protocol against 16 antibiotics. Phenotypic screening of ESBLs was undertaken using Vitek 2 compact system .100total positive results of *E. coli* isolates isolated from 399 from urine specimens (urine) the highest percentage of *E. coli* isolated from female urine sample (18.55%) while from male urine (6.52%), when performing of antibiotic susceptibility the rate highest resistance were ampicillin (99%), followed by cefazolin (98%), ceftazidime (97%), ceftriaxone (97%), and cefepime (96%), respectively in contract the highest effective antibiotic against *E. coli* were imipenem(98%), followed by ertapenem (98%), and nitrofurantion (97%) piperacilin/ tazobactem (90%) ESBL producer of *E. coli* 91 (91%) by using Vitek 2 system. Morbidity and mortality attributable to third-generation-cephalosporin resistant *E. coli* ESBL is significant. If prevailing resistance trends continue, high societal and economic costs can be expected. Better management of infections caused by resistant *E. coli* is becoming essential.

Keywords: *E. coli*, Urinary tract infection, antibiotics resistance, ESBL;

INTRODUCTION

Bacteriuria is the presence of these bacteria in the UT when the patient has no physical symptoms normally associated with UTI such as pain, frequency and urgency. Under these conditions, *E. coli* strains exist in an asymptomatic carrier state without any obvious symptoms of UTI .*E. coli* strains that colonize the UT may ascend towards bladder to cause cystitis, which is usually associated with the classic symptoms of UTI, i.e. pain, frequency, and urgency. UTI can proceed from the bladder, via the ureters to the kidney, to cause pyelonephritis, with the possibility of causing irreversible kidney damage leading to kidney failure and death [1]Imaging in those less than six month old or who have unusual Cystitis findings [2]or bladder infection, is the most common urinary tract infection. It occurs in the lower urinary tract (the bladder and urethra) and nearly always in women. In most cases, the infection is brief and acute and only the surface of the bladder is infected. Deeper layers of the bladder may be harmed if the infection becomes persistent, or

chronic, or if the urinary tract is structurally abnormal. Pyelonephritis(Kidney Infection). Sometimes the infection spreads to the upper tract (the ureters and kidneys).They are the most common cause of hospital acquired infections accounting for approximately 40%. Rates of asymptomatic bacteria in the urine increase with age from two to seven percent in women of child bearing age to as high as 50% in elderly women in care homes. Rates of asymptomatic bacteria in the urine among men over 75 are between 7-10%.Asymptomatic bacteria in the urine occurs in 2% to 10% of pregnancies [3].Recurrent urinary infection either through relapse or reinfection is common in patients who experience complicated urinary infection. *E.coli* is the cause of 80-85% of urinary tract infections, with *Staphylococcus saprophyticus* being the in 5-10% [4] Extended-spectrum Betalactamases (ESBLs) are a heterogeneous group of plasmid mediated bacterial enzymes that confer significant resistance to oxyimino cephalosporin and monobactam antimicrobials [5]. Four molecular classes of these enzymes

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have been described (A–D) and include metal dependent (Zn²⁺-requiring; class B) and metal-independent (active site serine; classes A, C, and D) beta – lactamases [6]. Prevalence of ESBL producing strains also varies from one geographical region to another paralleling the misuse or overuse of beta- lactam drugs. Resistance to betalactam antibiotics is mainly due to the enzymes that hydrolyse the betalactam ring of the antibiotics, making it lifeless [7]. Antibiotic resistance is more frequently originated in gram negative rather than gram positive isolates. The British medical journal Lancet warns that the drug resistant bacteria could spark a pandemic. Another alarming finding is that 3-5% of population carries ESBL forming bacteria in the intestine without knowing it. Even modern antibiotics are completely ineffective against these drug resistant bacteria. Recently however the pressure of increasing drug resistance has renewed efforts to discover novel chemical classes of both naturally occurring and synthetic bacterial compounds of antibiotic surveillance program and it is also especially concerned with prevalence of beta-lactamase enzyme in urinary tract gram negative isolates [8].

MATERIALS AND METHODS

Sample Collection

A total of (399) samples were collected from (urine). After collection all bacterial isolates were subjected to a series of confirming tests. Clinical samples were collected from patients attending Rizzgary hospital in Erbil city during the period August to February 2017. Clean-Catch midstream urine of the patients was collected in a sterile tube (5ml) and immediately transported to the laboratory. Guidelines for proper specimen collection were given to all patients. A positive urine culture is based on the growth of bacteria at a high number of colony forming units (CFUs). Urine culture results should be interpreted in conjunction with clinical symptoms of urinary tract infection. For clean-catch urine samples, a positive urine culture as indicated by the growth of bacteria greater than 100,000 CFU/ mL is suggestive of UTI liability of results is determined by the quality of the specimen and specimen collection, transport, and handling to the laboratory.

Identification of Bacteria

This includes shape of the cell and reaction to gram stain the *E.coli* color is red or pink. Smears were prepared from isolated bacterial

culture, stained with gram stain and examined under light microscope using oil immersion objective lens. In order to obtain maximal yield, specimens were inoculated to several culture media after incubation overnight at 37C°, the cultural characteristic of isolated bacterial colonies were identified [10] as following

Vitek 2 Compact System

The newly redesigned colorimetric Vitek 2 compact system, with updated advanced expert system (AES) (bioMerieux, Marcy l'Etoile, France) was evaluated for its accuracy and rapidity to identify clinical isolates and to detect several antimicrobial resistances [11]. Principles of the Vitek 2 is an automated microbiology system utilizing growth-based technology. This system accommodates the colorimetric reagent cards that are incubated and interpreted automatically. Overall, the Vitek 2 gave 95.8% of compatibility with the reference API strips (bioMerieux) in the identifications (ID) of the Gram- positive cocci (GPC), Gram-negative rods (GNR), and yeasts. The accuracy was finally estimated to 98.3% through additional confirmatory tests. Also, > 90% of identifications of GPC and GNR were obtained within 7 hours of incubation. The most resistant isolates were identified within 12 hours of incubation. In conclusion, the new colorimetric Vitek 2 compact system with AES greatly improved its accuracy in species identification and detection of antimicrobial resistances, and it will be highly acceptable to clinical microbiology laboratory function [12] while inserting the transfer tube into the corresponding suspension tube and the filled cassette was placed manually after reading the barcode of the cards.

Antimicrobial Susceptibility Test by Vitek 2 System

The system includes an AES that analyzes minimum inhibitory concentration (MIC) patterns and detects phenotypes for most organisms tested. This helps optimize laboratory efficiency for lean laboratory management. Rapid results allow clinicians to discontinue empiric therapy and prescribe targeted therapy, resulting in improved patient outcomes and enhanced antibiotic stewardship [13]. With its ability to provide accurate "fingerprint" recognition of bacterial resistance mechanisms and phenotypes, the AES is a critical component of Vitek 2 technology [14]. The Vitek 2 card contains 64 micro wells. Each well contains identification substrates or antimicrobial. Vitek 2 offers a

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comprehensive menu for the identification and antibiotic susceptibility testing of organisms [15]. The Vitek 2 test card is sealed, which minimizes aerosols, spills, and personal contamination. Disposable waste is reduced by more than 80% over microtiter methods.

RESULTS AND DISCUSSION

The Incidence of *E. Coli* in Urine Specimens

One hundred (25.06%) infected sample of urine with *E.coli* were isolated from 399(100%) sample and 299(74.90%) uninfected sample were isolated from urine. as in table (1) and in figure (1).

A total of (399) samples were collected from urine sample. After collection all bacterial isolates were subjected to a series of confirming tests All *E. coli* isolated and identified by using microscopically, macroscopically, biochemical tests, Api 20 E and Vitek 2 Results showed that only (100) isolates were indicated as *E. coli* The results in table (1) showed that we are collected a total of 100(25.06%) *E. coli* positive out of (399) urine samples, our study lower than Astal [16] from Iran who were collected (553) sample, 326(58.95%) was *E. coli* lower than reported by Stamm [17]from Iran who was found (58.95%) of *E. coli* causing urinary tract infection.

Table1. The incidence of *E.coli* in urine specimens.

Patients	Urine samples	
	NO.	%
Infected	100	25.06%
Uninfected	299	74.90%
Total	399	100%

Distribution of *E. Coli* in Relation with Gender in Urine Specimens

After the interpretation of the data we found that the prevalence of *E.coli* was analyzed according to persons gender among the 100specimens of

infected patient 74 (18.55%) were from females and 26 (6.52%) from males. In the present study the higher rate of *E.coli* was found in females compared to males on the other hand the highest rate of *E.coli* causing UTI was found in female urine (18.55%) compared to (6.52%)in male urine. And uninfected sample was 299 patient, 222(55.60%)was female and 77 (19.20%) was male from total of uninfected samples as in table (2)&in figure (4).

Table2. Distribution of *E. coli* in relation with gender in urine specimens.

Patients	Urine samples			
	Infected		Non infected	
	NO.	%	NO.	%
Female	74	18.55%	222	55.60%
Male	26	6.52%	77	19.20%
Total	100	25.07	299	100%

After the interpretation of the data in table(2) the prevalence of *E. coli* was analyzed according to person's gender among the 100 positive growth 74 (18.55%) were from females and 26(6.52%) from males. In the present study the higher rate of *E. coli* was found in females compared to males. Our results agree with that recorded by Stratchounski [18] who found that (75.5%) of *E. coli* isolates are females and (24.5%) was male in Iran country. In our study, the higher rate of *E. coli* was found in females urine compared to males urine. The higher incidence of urinary tract infections in females is due to unique anatomical features of the female genitourinary tract, which include a shorter urethra and the more proximal location of the urethral meat us to the anus makes it easy for bacteria to ascend in the urinary tract.

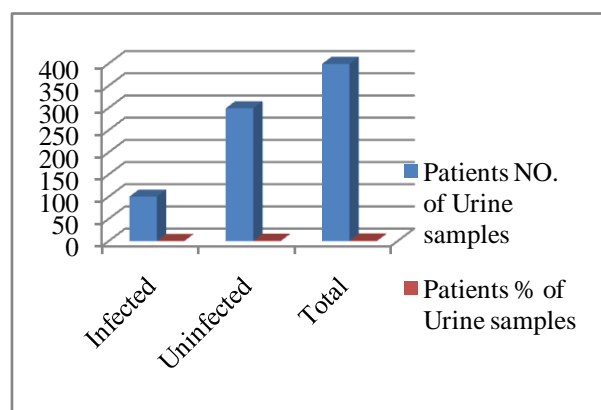


Figure1. The incidence of *E. coli* in urine specimens

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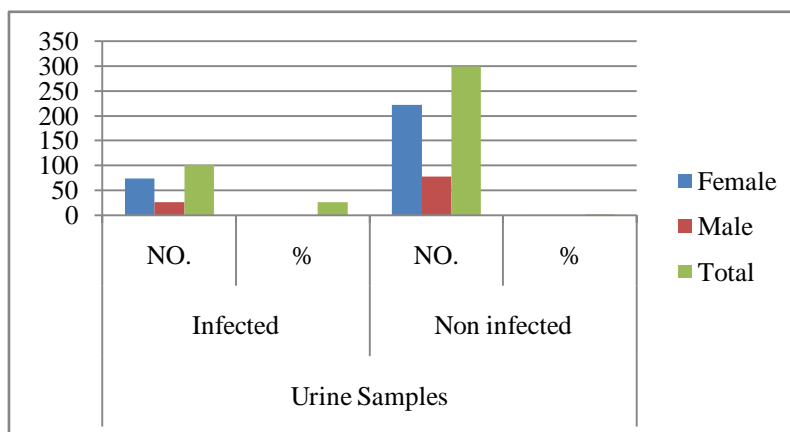


Figure4. Distribution of *E. coli* in relation with gender in urine specimens

Table3. Antimicrobial susceptibility tests for *E.coli*

Antibiotic	NO of Resistance	% of Resistance	NO of Sensitive	% of Resistance
AM	99	99%	1	1%
AMC	29	29%	71	71%
AM-SUM	61	61%	39	39%
P-TZB	10	10%	90	90%
CFZ	98	98%	2	2%
CAZ	97	97%	3	3%
CRO	97	97%	3	3%
FEP	96	96%	4	4%
ETP	2	2%	98	98%
IPM	2	2%	98	98%
GM	51	51%	49	49%
TOB	37	37%	63	63%
CIP	53	53%	47	47%
LVX	53	53%	47	47%
NIT	3	3%	97	97%
TMP-CMX	72	72%	28	28%

Antimicrobial Susceptibility Tests for *E.Coli*

E.coli isolates were screened for their resistance to sixteen widely used antibiotics (ampicillin, amoxicillin/clavulanic acid, ampicillin/sulbactam, piperacillin/tazobactam, cefazolin, ceftazidime, ceftriaxone, cefepime, ertapenem, imipenem, gentamicin, tobramycin, ciprofloxacin, levofloxacin, trimethoprim/sulfamethoxazole, nitrofurantoin). From the table (3) it is obvious that *E.coli* isolates showed high resistance (99%) to ampicillin, (98%) to cefazolin, (97%) for ceftazidime & ceftriaxone & (96%) cefepime. About sensitivity the high sensitive (98%) to imipenem & ertapenem, (97%) to nitrofurantoin & (90%) for piperacillin/ tazobactam as in figure(5). In our study as we mentioned in table(3) & in Table (4) the percentage of antibiotic resistant in *E. coli* which the results revealed high resistant to ampicillin 99% similar results were recorded by Sharif [19] they found that resistant of *E. coli* infections to ampicillin

92.4 % respectively. Hooton, [20] stated that no *E. coli* isolates were sensitive to ampicillin, also our result agreed with other studies done by Zhanel [21] they found that ampicillin resistance percent was 92.4% among *E. coli* isolated. The second most frequent resistance observed in this work was cefazolin 98% disagree results obtained by Mandell *et al.*, [22] who reported that 25.2% of *E. coli* isolates were resistant to cefazolin. All isolates were resistance to ceftazidime and ceftriaxone 97% & for cefepime 96% was reported in our study its agreement with results obtained by Foxman [23] The most resistant antibiotics against *E. coli* infection isolates were ampicillin, cefazolin, ceftazidime, Ceftriaxone these results do agree with that researched by Andrade [24] stated that *E. coli* mostly resistance against the ampicillin, cephalothin, tetracycline, while, also our result consistent with that study done by Abu Shaqra [25] they found that 95.1% of *E. coli* isolates were resistant to ampicillin In Iran. In present

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study, the most sensitive antibiotics against *E. coli* infection isolates were piperacilin/tazobactam, ertapenem, imipenem & nitrofurantion as in table (4) these result do disagree with that researched by Russel *et al.*, [26] stated that the gentamicin, ceftriaxone, amikacin, and cefotaxime, ciprofloxacin and tobramycin are effective for *E. coli* infection, on the other hand our result was disagree with that study done by Ronald [27]they found that 90.5% of *E. coli* isolates were sensitive to gentamicin, ceftriaxone, amikacin. This high rate of resistant to ampicillin, ceftazidime, ceftriaxone and cefepime may reflect the fact that these are the

most commonly prescribed antibiotics in hospital and also the most easily available in the community without prescription and so subject to abuse and misuse of antibiotic. This high resistant may be also due to the spontaneous and there are no control on take the drugs, and about 50% of it given to outpatients without physicians prescription are from outside of hospital, as well as the occurrence of any infection in the organ of patient, they taking antibiotics without culturing and determination of antibiotic susceptibility for its side effect, the emergence of different types of antibiotics.

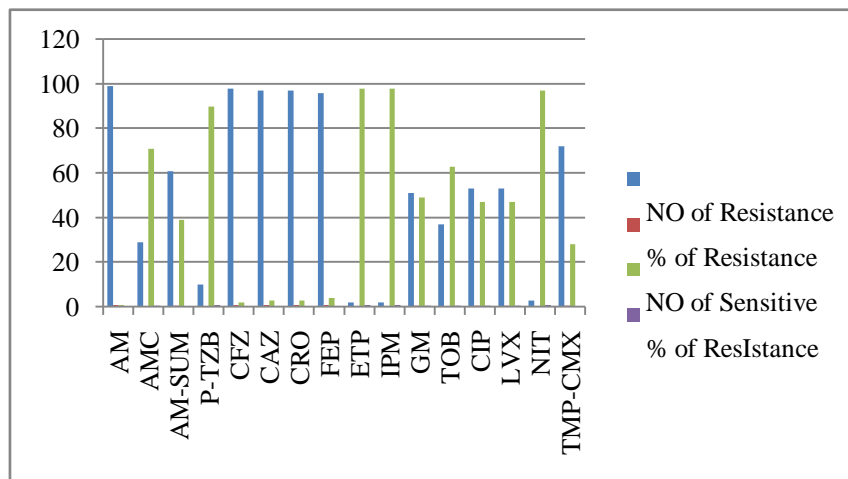


Figure3-5. Antimicrobial susceptibility tests for *E.coli*

Antibiotics Resistance Patterns and ESBL Producing for *E. Coli* Isolates

The antibiotic resistance patterns are shown in table (4). The bacterial isolates revealed remarkable variation in their resistance to antibiotics used, but in general most isolates of *E.coli* were multidrug resistance to more than four antibiotics, most isolates 91(91%) of *E. coli* ESBL producer.

E.coli remarkable variable to their resistance to antibiotic use, but in general most isolates of Gram negative bacteria were multidrug resistance to more than four antibiotics. Multidrug resistance is defined as insensitivity or resistance of microorganisms to the administrated antimicrobial medicines which are structurally unrelated and have different molecular targets despite earlier sensitivity to it. Studies from WHO report have shown very high rate of resistance in bacteria such as *E. coli* against antibiotics such as cephalosporin and fluoroquinolones, *Klebsiella pneumonia* against cephalosporin and carbapenems [28].

Antibiotics Resistance Patterns and ESBL Producing for *E. Coli* Isolates

Production of ESBL has been reported in virtually all species of Enterobacteriaceae, which greatly complicates the therapy of infections caused by these organisms. However, the frequency of isolates producing AmpC β -lactamases, especially plasmid mediated AmpC (pAmpC), is largely unknown. These β -lactamases confer resistance to broad spectrum cephalosporins and aztreonam, a multidrug-resistant (MDR) profile. The result in table (4) showed that 91 (91%) samples of *E.coli* producers of ESBL from total (100) samples of *E.coli* our result were compared with other result and found that higher than study recorded by Forbes [29] who found that from total (2725) samples of *E.coli* 162(6%) samples have ESBL positive from Spain country & higher than result (58.06%) reported in India by Anantha krishanan [30].

Our result supports the evidence that *E.coli* yielded the highest percentage of ESBL producers and all isolates of *E.coli* from all urine

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samples were generally resistant to the ampicillin. *E.coli* now became the more common in multidrug resistance isolate and the problem of beta lactamases is now more prevalent in Enterobacteriaceae. ESBL producers are more susceptible to ceftriaxone. The ESBL enzymes are plasmid-mediated enzymes capable of hydrolyzing and inactivating a wide variety of β -lactams, including third generation cephalosporin, penicillin and aztreonam. These enzymes are the result of mutations of TEM-1 and TEM-2 and

SHV-I. All of these β -lactamase enzymes are commonly found in the Enterobacteriaceae family. Normally, TEM-1, TEM-2 and SHV-1 enzymes confer high level resistance to early penicillin and low level resistance to first generation cephalosporin. Widespread use of third generation cephalosporins and aztreonam is believed to be the major cause of the mutations in these enzymes that has led to the emergence of the ESBLs Chaudhary [31].

Table4. Antibiotics resistance patterns and ESBL producing for *E.coli* isolates

Patient NO.	ESBL	Antibiotic																resistant NO	resistance%	sensitive NO	sensitive %
		AM	AMC	AM-SUL	P-TZB	CFZ	CAZ	CRO	FEP	FTP	IPM	GMI	TOB	CIP	LVX	NIT	TMP-SMX				
1	+	R	I	R	S	R	R	R	R	S	S	R	R	R	R	S	R	11	68.75	5	31.25
2	+	R	R	R	I	R	R	R	R	S	S	R	R	R	R	R	R	13	81.25	3	18.75
3	+	R	I	R	S	R	R	R	R	S	S	R	I	R	R	S	R	10	62.5	6	37.5
4	+	R	S	R	S	R	R	R	R	S	S	R	I	S	S	S	R	8	50	8	50
5	-	R	R	R	R	R	R	R	R	S	S	R	R	R	R	S	S	12	75	4	25
6	+	R	S	S	S	R	R	R	R	S	S	S	S	S	S	S	S	5	31.25	11	68.75
7	+	R	S	S	S	R	R	R	R	S	S	S	S	S	S	S	R	6	37.5	10	62.5
8	-	R	R	R	R	R	R	R	R	S	S	S	R	R	R	S	R	12	75	4	25
9	+	R	S	S	S	R	R	R	R	S	S	S	S	R	R	S	R	8	50	8	50
10	+	R	I	R	S	R	R	R	R	S	S	S	S	S	S	S	S	6	37.5	10	62.5
11	+	R	I	R	S	R	R	R	R	S	S	S	R	R	R	S	R	10	62.5	6	37.5
12	+	R	I	R	S	R	R	R	R	S	S	R	R	R	R	S	S	10	62.5	6	37.5
13	+	R	S	S	S	R	R	R	R	S	S	S	S	S	S	S	S	5	31.25	11	68.75
14	+	R	I	R	I	R	R	R	R	S	S	R	R	R	R	S	R	11	68.75	5	31.25
15	+	R	S	R	S	R	R	R	R	S	S	R	I	S	S	S	S	7	43.75	9	56.25
16	+	R	S	S	S	R	R	R	R	S	S	S	S	S	S	S	S	5	31.25	11	68.75
17	+	R	R	R	R	R	R	R	R	S	S	R	R	R	R	S	R	13	81.25	3	18.75
18	+	R	S	I	S	R	R	R	R	S	S	R	S	S	S	R	R	8	50	8	50
19	+	R	R	R	S	R	R	R	R	S	S	R	R	R	R	S	R	12	75	4	25
20	+	R	R	R	R	R	R	R	R	R	R	I	R	R	R	I	S	13	81.25	3	18.75
21	+	R	R	R	S	R	R	R	R	S	S	R	R	R	R	S	R	12	75	4	25
22	+	R	S	S	S	R	R	R	R	S	S	S	S	S	S	S	S	5	31.25	11	68.75

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23	+	R	S	S	S	R	R	R	R	S	S	S	S	S	S	S	S	5	31.25	11	68.75
24	+	R	R	R	I	R	R	R	R	S	S	R	S	S	S	S	R	9	56.25	7	43.75
25	+	R	S	I	S	R	R	R	R	S	S	S	S	I	I	R	R	7	43.75	9	56.25
26	+	R	S	I	S	R	R	R	R	S	S	R	S	S	S	S	S	6	37.5	10	62.5
27	-	R	R	R	I	R	R	R	S	S	S	R	I	R	R	S	R	10	62.5	6	37.5
28	+	R	S	S	S	R	R	R	R	S	S	S	S	S	S	S	R	6	37.5	10	62.5
29	-	R	R	R	R	R	R	R	R	S	S	R	R	R	R	S	R	13	81.25	3	18.75
30	+	R	S	S	S	R	R	R	R	S	S	S	S	S	S	S	R	6	37.5	10	62.5
31	+	R	S	S	S	R	R	R	R	S	S	S	S	S	S	S	S	5	31.25	11	68.75
32	+	R	I	R	S	R	R	R	R	S	S	S	R	R	R	S	R	10	62.5	6	37.5
33	+	R	R	R	I	R	R	R	R	S	S	R	R	R	R	S	R	12	75	4	25
34	+	R	S	I	S	R	R	R	R	S	S	S	S	S	S	S	R	6	37.5	10	62.5
35	+	R	S	I	S	R	R	R	R	S	S	S	S	I	I	S	R	6	37.5	10	62.5
36	+	R	R	R	R	R	R	R	R	S	S	S	S	R	R	S	S	10	62.5	6	37.5
37	+	R	S	I	S	R	R	R	R	S	S	R	S	S	S	S	R	7	43.75	9	56.25
38	+	R	R	R	S	R	R	R	R	S	S	R	R	R	R	S	S	11	68.75	5	31.25
39	+	R	I	R	S	R	R	R	R	S	S	R	R	R	R	I	R	11	68.75	5	31.25
40	+	R	S	R	S	R	R	R	R	S	S	R	I	S	S	S	S	7	43.75	9	56.25
41	+	R	S	S	S	R	R	R	R	S	S	S	S	R	R	S	R	8	50	8	50
42	+	R	S	S	S	R	R	R	R	S	S	S	S	R	R	S	R	8	50	8	50
43	+	R	R	R	I	R	R	R	R	S	S	R	R	R	R	I	S	11	68.75	5	31.25
44	+	R	S	S	S	R	R	R	R	S	S	S	S	S	S	S	R	6	37.5	10	62.5
45	+	R	R	R	S	R	R	R	R	S	S	I	S	I	I	S	R	8	50	8	50
46	+	R	S	I	S	R	R	R	R	S	S	S	S	S	S	S	S	5	31.25	11	68.75
47	+	R	S	S	S	R	R	R	R	S	S	S	S	R	R	S	R	8	50	8	50
48	+	R	R	R	S	R	R	R	R	S	S	S	I	R	R	S	R	10	62.5	6	37.5
49	+	R	S	S	S	R	R	R	R	S	S	R	S	R	R	S	R	9	56.25	7	43.75
50	+	R	S	R	S	R	R	R	R	S	S	R	I	S	S	S	S	7	43.75	9	56.25
51	+	R	S	S	S	R	R	R	R	S	S	S	S	R	R	S	R	8	50	8	50
52	+	R	R	R	I	R	R	R	R	S	S	R	R	R	R	S	R	12	75	4	25
53	+	R	I	R	S	R	R	R	R	S	S	R	I	S	S	I	R	8	50	8	50
54	+	R	S	I	S	R	R	R	R	S	S	S	S	I	I	S	R	6	37.5	10	62.5
55	+	R	S	S	S	R	R	R	R	S	S	S	S	S	S	S	S	5	31.25	11	68.75
56	+	R	I	R	S	R	R	R	R	S	S	S	S	S	S	S	R	7	43.75	9	56.25
57	+	R	R	R	I	R	R	R	R	S	S	R	I	S	S	S	R	9	56.25	7	43.75
58	+	R	R	R	S	R	R	R	R	S	S	R	R	R	R	S	R	12	75	4	25
59	+	R	S	R	S	R	R	R	R	S	S	R	I	S	S	S	R	8	50	8	50
60	+	R	R	R	S	R	R	R	R	S	S	S	R	R	R	S	R	11	68.75	5	31.25
61	+	R	S	R	S	R	R	R	R	S	S	S	S	S	S	S	R	7	43.75	9	56.25
62	+	R	S	R	S	R	R	R	R	S	S	S	S	S	S	I	R	7	43.75	9	56.25

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63	+	R	R	R	I	R	R	R	R	S	S	S	S	S	S	R	8	50	8	50	
64	+	R	R	R	I	R	R	R	R	S	S	R	S	S	S	R	9	56.25	7	43.75	
65	+	R	R	R	S	R	R	R	R	S	S	I	I	S	S	I	R	8	50	8	50
66	+	R	S	R	S	R	R	R	R	S	S	R	I	R	R	I	R	10	62.5	6	37.5
67	-	R	I	R	S	R	R	R	R	S	S	R	R	S	S	S	R	9	56.25	7	43.75
68	+	R	I	R	S	R	R	R	R	S	S	R	R	R	R	S	R	11	68.75	5	31.25
69	+	R	R	R	R	R	R	R	R	S	S	R	R	R	R	S	S	12	75	4	25
70	+	R	I	R	S	R	R	R	R	S	S	R	R	R	R	S	R	11	68.75	5	31.25
71	+	R	S	I	S	R	R	R	R	S	S	S	S	S	S	S	R	6	37.5	10	62.5
72	+	R	I	R	S	R	R	R	R	S	S	R	R	R	R	S	S	10	62.5	6	37.5
73	+	R	R	R	S	R	R	R	R	S	S	R	R	R	R	S	R	12	75	4	25
74	+	R	I	R	S	R	R	R	R	S	S	R	I	R	R	S	R	10	62.5	6	37.5
75	+	R	I	R	I	R	R	R	R	S	S	R	R	S	S	S	R	9	56.25	7	43.75
76	+	R	I	R	S	R	R	R	R	S	S	R	R	R	R	S	R	11	68.75	5	31.25
77	+	R	S	S	S	R	R	R	R	S	S	S	S	S	S	S	R	6	37.5	10	62.5
78	-	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	R	3	18.75	13	81.25
79	+	R	I	R	S	R	S	S	S	S	S	S	S	R	R	S	R	6	37.5	10	62.5
80	+	R	S	I	S	R	R	R	R	S	S	R	I	S	S	S	S	6	37.5	10	62.5
81	+	R	S	R	S	I	S	S	S	S	S	S	R	R	R	S	R	6	37.5	10	62.5
82	+	R	S	S	S	R	R	R	R	S	S	S	S	R	R	S	R	8	50	8	50
83	-	R	R	R	R	R	R	R	R	S	S	R	R	R	R	I	R	13	81.25	3	18.75
84	+	R	I	R	S	R	R	R	R	S	S	R	R	S	S	S	R	9	56.25	7	43.75
85	+	R	S	S	S	R	R	R	R	S	S	S	S	R	R	S	R	8	50	8	50
86	+	R	S	S	S	R	R	R	R	S	S	R	I	S	S	S	R	7	43.75	9	56.25
87	+	R	I	R	S	R	R	R	R	S	S	R	R	R	R	S	R	11	68.75	5	31.25
88	+	R	R	R	I	R	R	R	R	S	S	R	R	R	R	S	S	11	68.75	5	31.25
89	+	R	S	S	S	R	R	R	R	S	S	S	S	R	R	S	R	8	50	8	50
90	-	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	15	93.75	1	6.25
91	+	R	S	S	S	R	R	R	R	S	S	S	S	S	S	S	S	5	31.25	11	68.75
92	+	R	S	I	S	R	R	R	R	S	S	S	S	S	S	S	S	5	31.25	11	68.75
93	+	R	I	R	S	R	R	R	R	S	S	S	S	S	S	S	S	6	37.5	10	62.5
94	+	R	S	I	S	R	R	R	R	S	S	R	I	R	R	S	S	8	50	8	50
95	+	R	S	S	S	R	R	R	R	S	S	S	S	R	R	S	R	8	50	8	50
96	+	R	I	R	I	R	R	R	R	S	S	R	R	S	S	S	I	8	50	8	50
97	+	R	S	S	S	R	R	R	R	S	S	S	S	R	R	S	R	8	50	8	50
98	+	R	I	R	S	R	R	R	R	S	S	R	R	S	S	S	R	9	56.25	7	43.75
99	-	R	R	R	R	R	R	R	R	S	S	R	R	R	R	I	R	13	81.25	3	18.75
100	+	R	S	S	S	R	R	R	R	S	S	S	S	R	R	S	R	8	50	8	50

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