WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 12, 403-420. Research Article ISSN 2277– 7105

CO-AMOXICLAV RESISTANCE BY SOME BACTERIA SPECIES ISOLATED FROM HOSPITALS IN KHARTOUM STATE, SUDAN

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Article Received on
11 Oct 2015,

Revised on 01 Nov 2015,
Accepted on 21 Nov 2015

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ABSTRACT

The aim of this study is to assess the resistance of some species of bacteria against co-amoxiclav (amoxicillin+ calvulanic acid). The study was carried out on 66 samples (43 *S. aureus* 65.2%, 18 *E.coli* 27.3% and 5 *proteusspp* 7.6%), which have been collected and clinically isolated from different specimens (wound infection - UTI - blood - abscesses and others) from patients in four major hospitals in Khartoum state, Sudan (Zaitouna Hospital -Yastapshiroun Hospital - Al-amal Hospital and Soba Hospital). A susceptibility tests for three types of antibiotics (Co-amoxiclav, amoxicillin and methicillin) had been done using diffusion method. The results showed that 15.4% from the samples are sensitive to the co-amoxiclav, 1.9% intermediate and 82.7% were found to be resistant. It has been concluded that co-amoxiclav resistance is increasing rapidly and a special care should be given from the authorities to this increase and for prescribing this antibiotic with the importance of involving the susceptibility test before prescription, so as to control the resistance acquired by the bacteria against this antibiotic.

KEYWORDS: Co-amoxiclav, amoxicillin, methicillin, resistance.

1. INTRODUCTION

During the last decade, the debate on antimicrobial resistance has intensified. Resistance to antimicrobial drugs has increased, becoming a worldwide human and veterinary medicine concern, as resistance genes can be exchanged between animals and humans.[1]

In his Nobel Prize speech in 1945, Alexander Fleming, who discovered penicillin, warned that bacteria could become resistant to these remarkable drugs. Indeed, the development of each new antibacterial drug has been followed by the detection of resistance to it. The development of resistance is a normal evolutionary process for microorganisms, but it is accelerated by the selective pressure exerted by widespread use of antibacterial drugs. Resistant strains are able to propagate and spread where there is non-compliance with infections prevention and control measures.[2]

The global increase in resistance to antimicrobial drugs, including the emergence of bacterial strains that are resistant to all available antibacterial agents, has created a public health problem of potentially crisis proportions.[3]

Production of β-lactamase in Gram-negative bacteria was described in 1940 in *E. coli*.[4] In the 1960s, β-lactamase produced by Gram-negative bacteria was found to be different from the staphylococcal type, being located intracellularly and thus able to act against the β-lactam agents on their entry into the bacterium. The development of ampicillin and then amoxicillin, with their extended antimicrobial activity against Gram-negative organisms, meant that β-lactamase production in these organisms became an important clinical issue. In addition, the ability to produce β-lactamase was found to be transferable between *E. coli* and other species via plasmids during cell-to-cell contact, raising the possibility of transferring to species not previously known to produce β-lactamase.[5]

It was known that cloxacillin, flucloxacillin and other β-lactamasestable β-lactams competitively inhibit β-lactamase, but these agents were not potent enough against the desired range of target enzymes to sufficiently protect the broad-spectrum penicillins. Eventually, after a specific screening program by BRL, a β-lactam molecule produced by *Streptomyces clavuligerus* was discovered and found to be a potent inhibitor of β-lactamases, but with low antibacterial activity; this molecule was named clavulanic acid. The β-lactam ring of clavulanic acid irreversibly binds to the bacterial β-lactamase, thus inhibiting the enzyme and preventing it from binding to and inactivating β-lactam antibiotics. Clavulanate

has some effects on pathogenic bacteria regardless of β-lactamase production, although their clinical significance has not been established.[6]

The β-lactamase inhibiting properties of clavulanic acid were combined with the good oral absorption and potent broad-spectrum antimicrobial activity of amoxicillin in tablets containing amoxicillin trihydrate and potassium clavulanate. In this form, amoxicillin/clavulanate was first launched as Augmentin® in the UK in 1981 and subsequently throughout the world. Pediatric formulations and an intravenous formulation followed and are now also available in many countries worldwide.[7] As amoxicillin/clavulanate has been the most therapeutically useful combination, most studies of clavulanate in combination with antibacterials have been with amoxicillin.[8]

Amoxicillin/clavulanate was originally developed in response to the need for an oral broad spectrum antibiotic that covered β-lactamase producing pathogens. Amoxicillin/clavulanate retained the good activity of amoxicillin against β-lactamase negative strains, restored its activity against β-lactamase producing strains, such as in *S. aureus*, *E.coli* and *H. influenzae*, and extended its activity against *Klebsiella pneumonia* and the anaerobic *Bacteroides fragilis* (most strains of the latter produce β-lactamase).[9]

After mentioning of historical development for penicillin compound to avoid resistant, in this study we will shed alight on the percentage of some bacteria resistance co-amoxiclav which is the latest invented penicillin compound.

2. MATERIALS AND METHODS

2.1 Materials

Materials used in this research were divided into two categories; tools and equipments and chemicals and reagents.

2.1.1 Tools & Equipments

Different tools and equipments used in this project can be described as follow:

- Bottles
- Conical flasks
- Spatula
- Loop
- Test tubes

- Petri Dishes
- Autoclave
- Laminar Flow Cabinet
- Incubator
- Balance
- Vortex
- Digital Vernier
- Diffusion discs (Co-Amoxiclav - Amoxicillin - Methicillin)

2.1.2 Chemicals & Reagents

The chemicals and reagents used in this research can be explained as below:

- The chemicals and reagents used in this research can explained as below:
- Mannitol Salt Agar
- DNAase Agar
- Mueller Hinton Agar
- Peptone Water (Phosphate Buffer)
- MacConkey Agar
- EMB Agar
- Blood Agar
- Kovacs Reagent

2.2 Methods

2.2.1 Samples collection

Sixty-six samples were collected from 66 patients admitted in four different hospitals at Khartoum state, Sudan (Zaitouna Hospital – Yastapshiroun Hospital - Al-amal Hospital and Soba Hospital) during the period between 12/2014 to 04/2015. 39 patients (59.1%) were males while the others 27 (40.9%) were females. 6 patients (10.5%) were children, 17 patients (29.8%) were adults and 34 patients (59.6%) were seniors. Samples were isolated and diagnosed as *Staphylococcus aureus*, *E.coli* and *Proteus spp* in hospitals, collected from different specimens (table 1), cultured and diagnosed according the standard.

Table (1): Explaining specimens percentage

Type of sample	No of sample	Percentage %
URINE	25	37.9
WOUND SWAB	17	25.8
GROIN SWAB	7	10.6
NOSE SWAB	5	7.6
AXILLA SWAB	5	7.6
BLOOD	3	4.5
ABCESS	2	3.0
TIB CULTER	1	1.5
SPUTEM	1	1.5

2.2.2 Confirmation test

Staphylococcus aureus

cultured in Mannitol Salt Agar and DNAse Agar.

E.coli

cultured in MacConkey Agar, EMB agar and indole test.

Proteus spp

cultured in Mac Conkey Agar and Blood Agar.[10]

2.2.3 Susceptibility test method

Preparation of inoculum

A loop full of bacteria was transferred from plate culture to 5 ml of peptone water and mixed very well.

Preparation of test medium

50 ml of Mueller Hintone agar was prepared according to the manufacturer[11], sterilized in autoclave at 121℃ for 15 minutes and cooled to 40℃ after sterilization.

Susceptibility test

Inoculum was transferred to test medium and mixed carefully then 15 ml to 20 ml of medium was poured to each of the 3 sterile petri dishes. After solidification, antibiotic diffusion discs of co-amoxiclav, methicillin and amoxicillin (the test of quality control was done) applied on the surface of media by sterile forceps to test microorganism susceptibility to antibiotics. Petri dishes incubated at 37℃ for 24 hours. After incubation period the inhibition zones were measured by using calibrated digital Vernier with accuracy 0.00 mm and all readings were

repeated three times for each zone.[12] Results were compared with limit of zone diameter to identify the susceptibility (table 2).

Table (2): Explaining discs information and limit of zone diameter

	Interpretation			
Antibioticmcg	**Bacteria**	**Susceptible**	**Intermediate**	**Resistant**
Amoxicillin 10mcg	*S.aureus*	≥29mm	-	≤28mm
	Others	≥17mm	14-16mm	≤13mm
Co-amoxiclav 30mcg	*S.aureus*	≥20mm	-	≤19mm
	Others	≥18mm	14-17mm	≤13mm
Methicillin 5mcg	*S.aureus*	≥14mm	13-10mm	≤9mm
	-	-	-	-

2.2.4 Quality control test

For method validation the susceptibility test was conducted on co-amoxiclav against standard strain (NCTC10788) repeated 3 times for 3 days then relative standard deviation was calculated and compared with the United States Pharmacopoeia (USP-37) limits.

The above method conducted on amoxicilline and methicilline.

2.2.5 Statistical analysis

Microsoft excel 2010 was used, for each zone three reading were taken, then average, standard deviation, relative standard deviation, percentages of resistant samples and percentages of sensitive samples were calculated and plotted as charts.

3. RESULTS

In this study it was found that

- 43 of the collected samples were samples of *Staphylococcus aureus* (65.2%), 18 *E.coli* (27.3%) and 5 *Proteus spp* (7.6%).
- Antibiotic discs and method are conform according to result (tables 3.1.1-3.1.2-3.1.3).

- ***S.aureas***

34.9% of the collected samples were MRSA which are totally resistant to amoxicillin & co-amoxiclav[13] and the remaining samples were methicilline susceptible *S.aureas*, which showed resistance of 92.9% for amoxicilline and 82.9% for co-amoxiclav (chart 3.5.1-3.5.2).

- ***E.coli***

94.4% resistant to amoxicillin and 88.9% resistant to co-amoxiclav (chart 3.6.1).

- ***Proteus* spp**

No significant difference between amoxicillin and co-amoxiclav both of them is showing 80% resistance (chart 3.7.1).

3.1.1 Result of Quality Control Tests

3.1.2 Co-amoxiclav Against *S.aureus* NCTC10788.

CO-AMOXICLAV	Zone mm 1	Zone mm 2	Zone mm 3	AVG mm	STDEV	RSD%
TEST 1	28.59	28.91	28.54	**28.68**	0.20	0.70
TEST 2	30.92	30.92	30.87	**30.90**	0.03	0.09
TEST 3	29.55	29.43	29.37	**29.45**	0.09	0.31

Average of the 3 tests results	
AVERAGE mm	29.68
STDEV	1.13
RSD %	3.80

Co-amoxiclav discs and method are conform according to result above.

3.1.2 Methicillin Against *S.aureus* NCTC10788.

Antibiotic	Zone 1 mm	Zone 2 mm	Zone 3 mm	AVERAGE	STDEV	RSD %
Methicillin	33.15	33.17	32.96	33.09	0.12	0.35

Methicillin discs are conform according to result above.

3.1.3 Amoxicillin Against *S.aureus* NCTC10788.

Antibiotic	Zone 1 mm	Zone 2 mm	Zone 3 mm	AVERAGE	STDEV	RSD %
Amoxicillin	31.13	31.11	31.01	31.08	0.06	0.21

Amoxicillin discs are conform according to result above.

3.2 Result of All Samples Against Methicillin.

NO	Result 1	Result 2	Result 3	AVERAGE	STDEV	RSD%
S-1	0	0	0	**0**	0	0
S-2	0	0	0	**0**	0	0
S-3	28.44	28.7	28.88	**28.67**	0.22	0.77
S-4	24.54	24.42	24.37	**24.44**	0.09	0.36
S-5	28.48	28.91	28.35	**28.58**	0.29	1.03
S-6	6.51	6.64	6.58	**6.58**	0.07	0.99
S-7	29.36	29.2	29.05	**29.2**	0.16	0.53
S-8	26.93	27.04	26.92	**26.96**	0.07	0.25
S-9	25.18	25.3	25.32	**25.27**	0.08	0.3
S-10	0	0	0	**0**	0	0
S-11	29.81	29.74	29.71	**29.75**	0.05	0.17
S-12	0	0	0	**0**	0	0

S-13	23.99	23.93	23.96	**23.96**	0.03	0.13
S-14	0	0	0	**0**	0	0
S-15	6.94	7.02	6.88	**6.95**	0.07	1.01
S-16	13.12	13.87	13.43	**13.47**	0.38	2.8
S-17	25.21	25.38	25.44	**25.34**	0.12	0.47
S-18	0	0	0	**0**	0	0
S-19	18.6	18.69	18.4	**18.56**	0.15	0.8
S-20	9.7	9.6	9.53	**9.61**	0.09	0.89
S-21	23.59	23.61	23.35	**23.52**	0.14	0.62
S-22	27.55	27.2	27.58	**27.44**	0.21	0.77
S-23	0	0	0	**0**	0	0
S-24	32.96	32.81	32.91	**32.89**	0.08	0.23
S-25	34.01	34.16	34.22	**34.13**	0.11	0.32
S-26	32.71	32.48	32.7	**32.63**	0.13	0.4
S-27	29.2	29.23	29.2	**29.21**	0.02	0.06
S-28	21.12	21.29	21.07	**21.16**	0.12	0.55
S-29	9.04	9	9.11	**9.05**	0.06	0.62
S-30	0	0	0	**0**	0	0
S-31	0	0	0	**0**	0	0
S-32	8.17	8.36	8.2	**8.24**	0.1	1.24
S-33	10.03	10.07	10.11	**10.07**	0.04	0.4
S-34	9.24	9.13	9.36	**9.24**	0.12	1.24
S-35	21.71	21.78	21.51	**21.67**	0.14	0.65
S-36	27.8	27.82	27.85	**27.82**	0.03	0.09
S-37	0	0	0	**0**	0	0
S-38	24.74	24.56	24.92	**24.74**	0.18	0.73
S-39	0	0	0	**0**	0	0
S-40	36.91	36.94	36.99	**36.95**	0.04	0.11
S-41	28.11	28.26	28.09	**28.15**	0.09	0.33
S-42	21.31	21.35	21.54	**21.4**	0.12	0.57
S-43	0	0	0	**0**	0	0
S-44	12.64	12.58	12.7	**12.64**	0.06	0.47
S-45	0	0	0	**0**	0	0
S-46	24.84	24.67	24.53	**24.68**	0.16	0.63
S-47	30.11	30.28	30.16	**30.18**	0.09	0.29
S-48	26.98	26.88	27.12	**26.99**	0.12	0.45
S-49	0	0	0	**0**	0	0
S-50	9.34	9.11	9	**9.15**	0.17	1.9
S-51	0	0	0	**0**	0	0
S-52	7.47	7.49	7.4	**7.45**	0.05	0.63
S-53	21.52	21.56	21.69	**21.59**	0.09	0.41
S-54	7.26	7.33	7.14	**7.24**	0.1	1.33
S-55	0	0	0	**0**	0	0
S-56	8.1	8.23	8.17	**8.17**	0.07	0.8
S-57	0	0	0	**0**	0	0
S-58	0	0	0	**0**	0	0
S-59	0	0	0	**0**	0	0
S-60	29.12	29.2	29.05	**29.12**	0.08	0.26

S-61	0	0	0	**0**	0	0
S-62	7.44	7.73	7.15	**7.44**	0.29	3.9
S-63	10.19	10.58	10.41	**10.39**	0.2	1.88
S-64	0	0	0	**0**	0	0
S-65	9.79	9.9	9.83	**9.84**	0.06	0.57
S-66	7.88	7.74	7.99	**7.87**	0.13	1.59

3.3 Result of All Samples Against Amoxicillin.

NO	Result 1	Result 2	Result 3	AVERAGE	STDEV	RSD%
S-1	7.2	7.26	7.29	**7.25**	0.05	0.6
S-2	6.52	6.47	7.58	**6.86**	0.63	9.1
S-3	8.99	8.49	8.95	**8.81**	0.28	3.2
S-4	8.46	8.29	8.36	**8.37**	0.09	1
S-5	0	0	0	**0**	0	0
S-6	6.88	6.71	6.84	**6.81**	0.09	1.3
S-7	10.95	10.63	10.99	**10.86**	0.2	1.8
S-8	10.69	10.52	10.62	**10.61**	0.09	0.8
S-9	16.31	16.34	16.66	**16.44**	0.19	1.2
S-10	0	0	0	**0**	0	0
S-11	12.54	12.81	12.85	**12.73**	0.17	1.3
S-12	0	0	0	**0**	0	0
S-13	8.41	8.75	8.44	**8.53**	0.19	2.2
S-14	9.91	9.99	10.01	**9.97**	0.05	0.5
S-15	6.94	6.91	6.99	**6.95**	0.04	0.6
S-16	7.24	7.38	7.11	**7.24**	0.14	1.9
S-17	8.66	8.27	8.38	**8.44**	0.2	2.4
S-18	0	0	0	**0**	0	0
S-19	0	0	0	**0**	0	0
S-20	6.8	6.49	6.51	**6.6**	0.17	2.6
S-21	8.53	8.54	8.43	**8.5**	0.06	0.7
S-22	7.27	7.21	7.25	**7.24**	0.03	0.4
S-23	6.41	6.39	6.44	**6.41**	0.03	0.4
S-24	23.92	23.71	23.78	**23.8**	0.11	0.4
S-25	22.11	22.37	22.19	**22.22**	0.13	0.6
S-26	34.11	33.51	33.88	**33.83**	0.3	0.9
S-27	8.75	8.4	8.55	**8.57**	0.18	2
S-28	15.82	15.82	15.88	**15.84**	0.03	0.2
S-29	0	0	0	**0**	0	0
S-30	0	0	0	**0**	0	0
S-31	0	0	0	**0**	0	0
S-32	11.36	11.4	11.33	**11.36**	0.04	0.3
S-33	11.35	11.19	11.22	**11.25**	0.09	0.8
S-34	26.3	26.45	26.38	**26.38**	0.08	0.3
S-35	8.28	8.41	8.63	**8.44**	0.18	2.1
S-36	9.35	9.7	9.28	**9.44**	0.23	2.4
S-37	0	0	0	**0**	0	0
S-38	8.11	8.7	8.46	**8.42**	0.3	3.5
S-39	8.12	8.33	9.04	**8.5**	0.48	5.7

S-40	19.36	19.61	19.4	**19.46**	0.13	0.7
S-41	24.05	24.16	24.21	**24.14**	0.08	0.3
S-42	10.87	10.81	11.05	**10.91**	0.12	1.1
S-43	8.11	8.36	8	**8.16**	0.18	2.3
S-44	8.09	8.15	8.27	**8.17**	0.09	1.1
S-45	0	0	0	**0**	0	0
S-46	33.12	33.44	33.28	**33.28**	0.16	0.5
S-47	10.48	10.69	10.4	**10.52**	0.15	1.4
S-48	8.88	8.95	8.91	**8.91**	0.04	0.4
S-49	0	0	0	**0**	0	0
S-50	9.91	9.97	9.92	**9.93**	0.03	0.3
S-51	10.16	10.25	10.43	**10.28**	0.14	1.3
S-52	8.47	8.38	8.51	**8.45**	0.07	0.8
S-53	11.29	11.32	11.44	**11.35**	0.08	0.7
S-54	7.37	7.29	7.18	**7.28**	0.1	1.3
S-55	0	0	0	**0**	0	0
S-56	8.75	8.8	8.83	**8.79**	0.04	0.5
S-57	0	0	0	**0**	0	0
S-58	0	0	0	**0**	0	0
S-59	0	0	0	**0**	0	0
S-60	30.14	30.35	30.27	**30.25**	0.11	0.4
S-61	0	0	0	**0**	0	0
S-62	9.18	9.23	9.31	**9.24**	0.07	0.7
S-63	9.75	9.88	9.66	**9.76**	0.11	1.1
S-64	0	0	0	**0**	0	0
S-65	8	8.17	8.22	**8.13**	0.12	1.4
S-66	0	0	0	**0**	0	0

3.4 Result of All Samples Against Co-amoxiclav.

NO	Result 1	Result 2	Result 3	AVERAGE	STDEV	RSD%
S-1	7.82	7.43	7.58	7.61	0.2	2.59
S-2	0	0	0	0	0	0
S-3	7.55	7.34	7.5	7.46	0.11	1.47
S-4	7.22	7.01	7.23	**7.15**	0.12	1.74
S-5	9.51	9.22	9.23	**9.32**	0.16	1.77
S-6	6.83	6.92	7.01	**6.92**	0.09	1.3
S-7	10.3	10.63	10.99	**10.64**	0.35	3.24
S-8	10.94	10.26	10.24	**10.48**	0.4	3.8
S-9	17.32	17.11	17.47	**17.3**	0.18	1.05
S-10	0	0	0	**0**	0	0
S-11	11.29	11.26	11.22	**11.26**	0.04	0.31
S-12	8.31	8.54	8.44	**8.43**	0.12	1.37
S-13	8.24	8.33	8.03	**8.2**	0.15	1.88
S-14	7.93	7.86	7.94	**7.91**	0.04	0.55
S-15	7.04	7.12	7.18	**7.11**	0.07	0.99
S-16	7.67	7.81	7.74	**7.74**	0.07	0.9
S-17	11.81	11.93	11.88	**11.87**	0.06	0.51
S-18	0	0	0	**0**	0	0

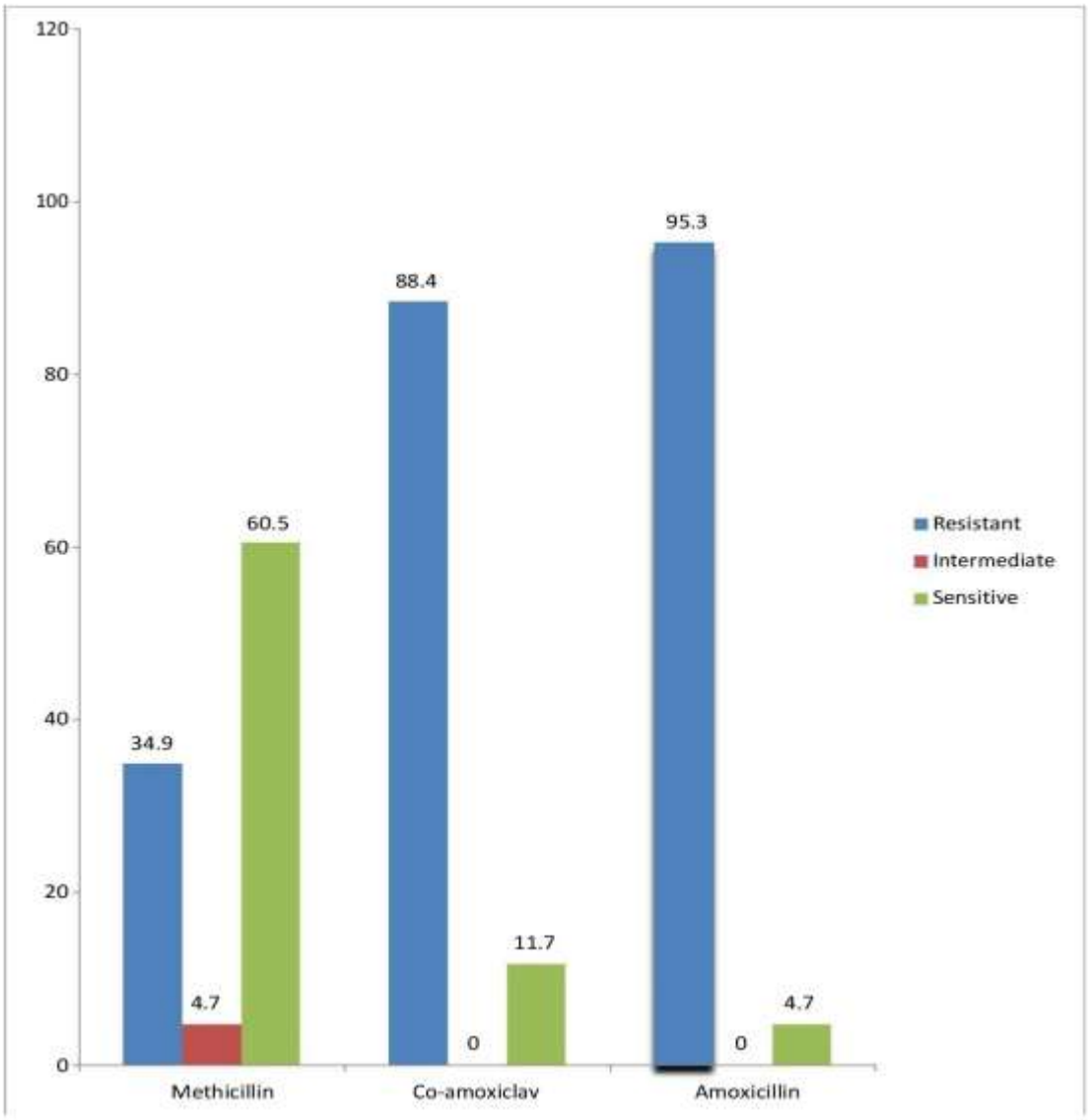
S-19	0	0	0	**0**	0	0
S-20	12.45	12.4	12.35	**12.4**	0.05	0.4
S-21	10.19	9.92	9.97	**10.03**	0.14	1.43
S-22	9.41	9.2	9.46	**9.36**	0.14	1.47
S-23	7.51	7.42	7.58	**7.5**	0.08	1.07
S-24	24.67	24.51	24.48	**24.55**	0.1	0.42
S-25	26.9	27.01	27	**26.97**	0.06	0.23
S-26	34.22	34.05	34.41	**34.23**	0.18	0.53
S-27	10.85	10.78	10.81	**10.81**	0.04	0.32
S-28	15.78	15.56	15.5	**15.61**	0.15	0.94
S-29	7.92	7.99	8.04	**7.98**	0.06	0.76
S-30	0	0	0	**0**	0	0
S-31	0	0	0	**0**	0	0
S-32	9.6	9.65	9.72	**9.66**	0.06	0.62
S-45	0	0	0	**0**	0	0
S-46	35.42	35.65	35.71	**35.59**	0.15	0.43
S-47	12.31	12.58	12.36	**12.42**	0.14	1.16
S-48	8.71	8.55	8.76	**8.67**	0.11	1.26
S-49	12.67	12.47	12.6	**12.58**	0.1	0.81
S-50	13.43	13.6	13.13	**13.39**	0.24	1.78
S-51	13.62	13.28	13.45	**13.45**	0.17	1.26
S-52	11.32	11.27	11.36	**11.32**	0.05	0.4
S-53	14.59	14.72	14.43	**14.58**	0.15	1
S-54	11.59	11.18	11.07	**11.28**	0.27	2.43
S-55	0	0	0	**0**	0	0
S-56	12.93	13.14	12.8	**12.96**	0.17	1.32
S-57	0	0	0	**0**	0	0
S-58	0	0	0	**0**	0	0
S-59	0	0	0	**0**	0	0
S-60	31.1	31.44	31.36	**31.3**	0.18	0.57
S-61	0	0	0	**0**	0	0
S-62	11.17	11.48	11.35	**11.33**	0.16	1.37
S-63	10.32	10.09	10.33	**10.25**	0.14	1.33
S-64	0	0	0	**0**	0	0
S-65	10.63	10.64	10.63	**10.63**	0.01	0.05
S-66	10.42	10.75	10.38	**10.52**	0.2	1.93

3.5 Result of Susceptibility Test to *S.Aureus*

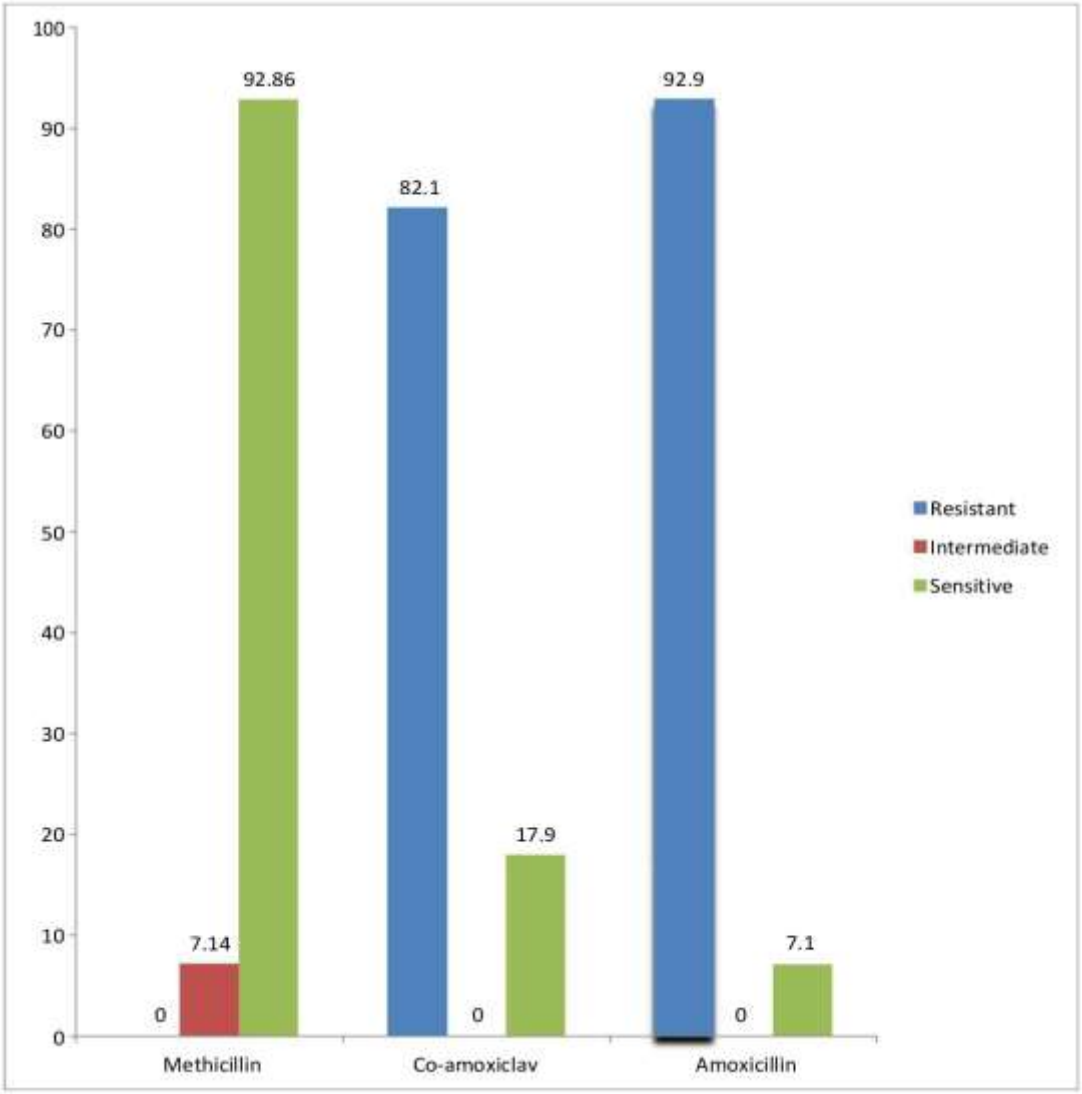
Note: The underlined results mean resistance.

No.	Amoxicillin	Co-amoxiclav	Methicillin
S-1	7.25	7.61	0.00
S-2	6.86	0.00	0.00
S-3	8.81	7.46	28.67
S-4	8.37	7.15	24.44
S-5	0.00	9.32	28.58
S-6	6.81	6.92	6.58
S-7	10.86	10.64	29.20

S-8	10.61	10.48	26.96
S-9	16.44	17.30	25.27
S-10	0.00	0.00	0.00
S-11	12.73	11.26	29.75
S-12	0.00	8.43	0.00
S-13	8.53	8.20	23.96
S-14	9.97	7.91	0.00
S-15	6.95	7.11	6.95
S-16	7.24	7.74	13.47
S-17	8.44	11.87	25.34
S-18	0.00	0.00	0.00
S-19	0.00	0.00	18.56
S-20	6.60	12.40	9.61
S-21	8.50	10.03	23.52
S-22	7.24	9.36	27.44
S-23	6.41	7.50	0.00
S-24	23.80	24.55	32.89
S-25	22.22	26.97	34.13
S-26	33.83	34.23	32.63
S-27	8.57	10.81	29.21
S-28	15.84	15.61	21.16
S-29	0.00	7.98	9.05
S-35	8.44	8.55	21.67
S-36	9.44	9.82	27.82
S-37	0.00	0.00	0.00
S-38	8.42	10.24	24.74
S-39	8.50	0.00	0.00
S-40	19.46	18.00	36.95
S-41	24.14	25.19	28.15
S-42	10.91	15.21	21.40
S-43	8.16	8.34	0.00
S-44	8.17	13.91	12.64
S-45	0.00	0.00	0.00
S-46	33.28	35.59	24.68
S-47	10.52	12.42	30.18
S-48	8.91	8.67	26.99



3.5.1 Chart Explaining Percentage of the Results of *S. aureus.*

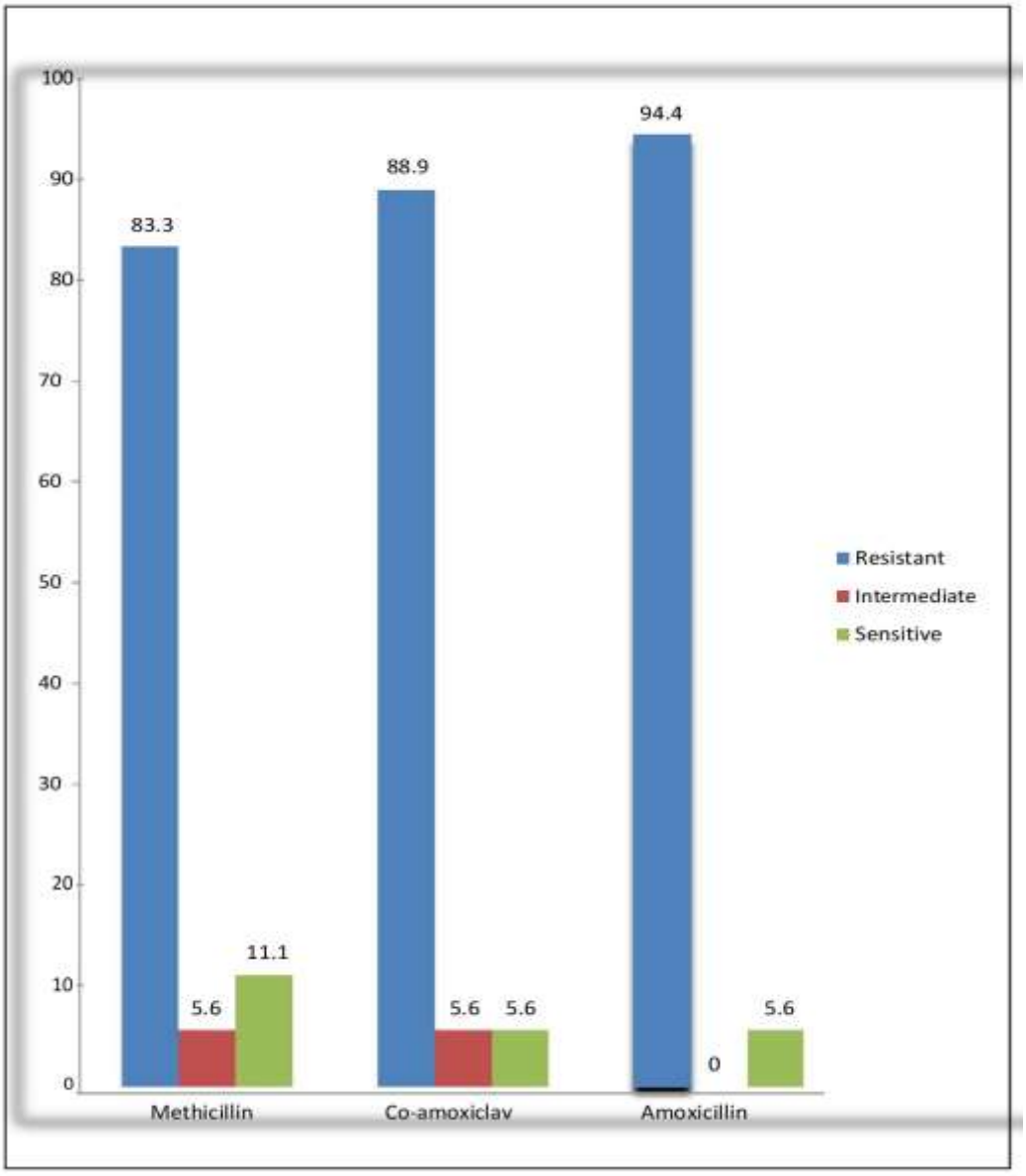


3.5.2 Results After Elimination of Methicillin Resistant Samples.

3.6 Result of Susceptibility Test to *E .coli.*

No.	Amoxicillin	Co-amoxiclav	Methicillin
S-30	0.00	0.00	0.00
S-31	0.00	0.00	0.00
S-32	11.36	9.66	8.24
S-33	11.25	14.60	10.07
S-51	10.28	13.45	0.00
S-52	8.45	11.32	7.45
S-53	11.35	14.58	21.59
S-54	7.28	11.28	7.24
S-55	0.00	0.00	0.00
S-56	8.79	12.96	8.17
S-57	0.00	0.00	0.00
S-58	0.00	0.00	0.00
S-59	0.00	0.00	0.00
S-60	30.25	31.30	29.12

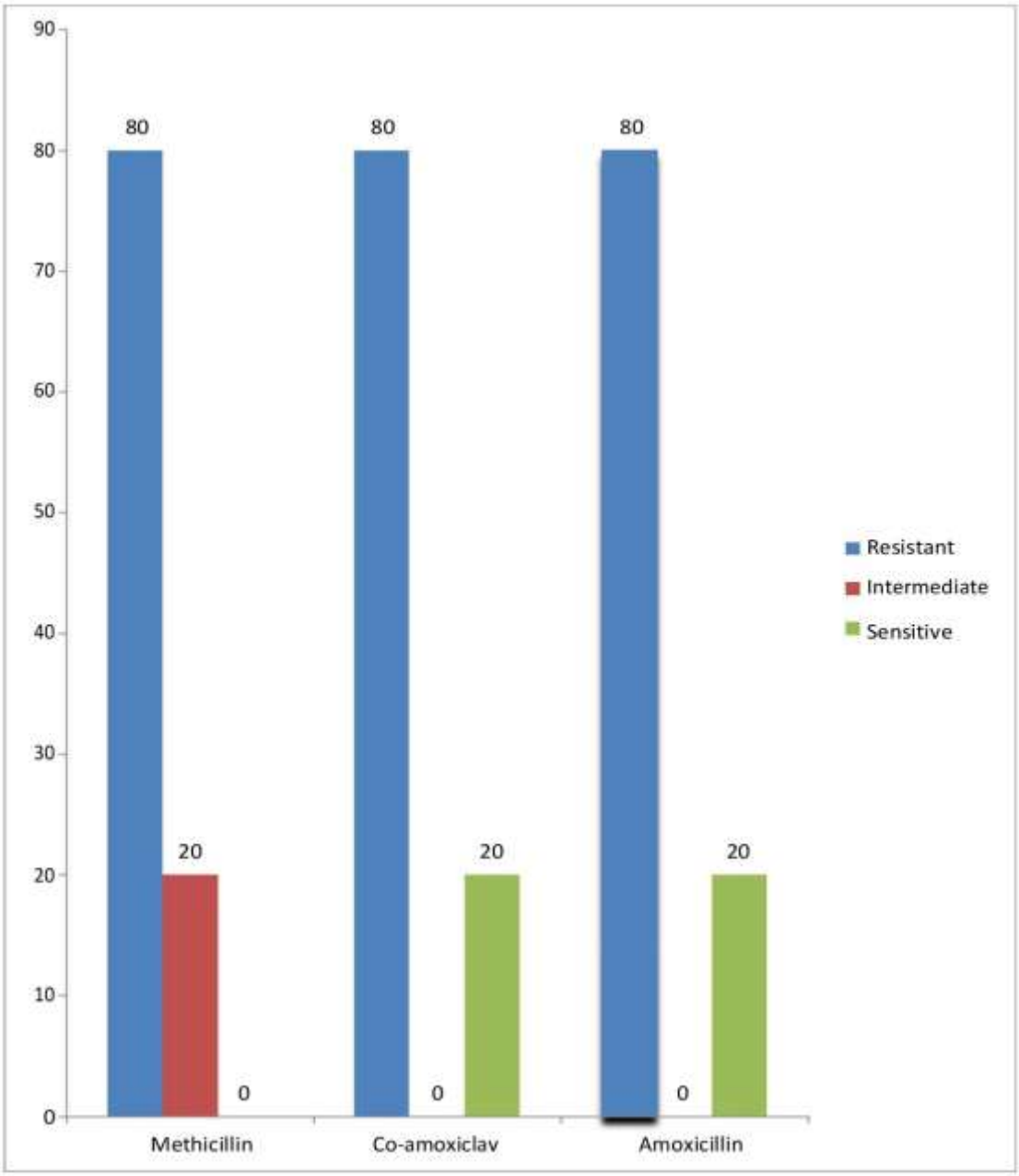
S-62	9.24	11.33	7.44
S-64	0.00	0.00	0.00
S-65	8.13	10.63	9.84
S-66	0.00	10.52	7.87



3.6.1 Chart Explaining Percentage of the Results of *E. coli.*

3.7 Result of Susceptibility Test to *Proteus spp.*

No.	Amoxicillin	Co-amoxiclav	Methicillin
S-34	26.38	25.98	9.24
S-49	0.00	12.58	0.00
S-50	9.93	13.39	9.15
S-61	0.00	0.00	0.00
S-63	9.76	10.25	10.39



3.7.1 Chart Explaining Percentage of the Results of *Proteus spp.*

4. DISCUSSION

These results show the extension of the closeness of the resistance between co-amoxiclav and amoxicillin although co-amoxicalv was developed to overcome amoxicillin resistance.

In 2013 the percentage of resistance to co-amoxiclav in Omdurman Teaching Hospital was found to be 64%[14], now in 2015 in Khartoum State hospitals the resistance to co-amoxiclav reached 82.7% which is considered the highest result obtained compared to the previous results. This may be mainly due to the abuse of co-amoxiclav.

5. CONCLUSION

This study is showing that there is a highly increasing resistance toward co-amoxiclav molecule even by *S. aureas* which is considered commonly susceptible. This also appeared with *E. coli* and *Proteus spp* which their acquired resistance may be a serious problem.

The percentage of the resistance in this study represents the highest one (82.7%), compared to all the previous studies; this may be highly attributed to misuse of antibiotics and to the prescription of the antibiotics without prior microbial sensitivity tests.

6. ACKNOWLEDGEMENT

The authors are greatly thankful to Azal Pharmaceutical Industries Co. Ltd. for providing raw materials, reagents as well as analysis facilities and also very grateful to the International University of Africa and to all who helped in this study, especially to Dr. Sameer Ghorashi.

7. REFERENCES

1. Liberato, R. B., Canga. A. G. and Martin. P. T. (2011). Amoxicillin and Amoxicillin-clavulanic Acid Resistance in Veterinary Medicine – The Situation in Europe: A Review, Veterinarni Medicina, 2011; 56(10): 473–485.
2. World Health Organization (WHO). Global Report, 2014.
3. Hogg, S. Essential Microbiology. John Wiley & Sons Ltd, England, 2005; ISBN 0 471 497533, P358.
4. Abraham, E. P. and Chain, E. (An enzyme from bacteria able to destroy penicillin). Nature, 2014; 146: 837.
5. Datta, N. and Kontomichalou. (Penicillinase synthesis controlled by infectious R factors in Enterobacteriaceae). Nature, 1965; 208: 239–241.
6. Finlay, J., Miller, L. and Poupard, J. A. (A review of the antimicrobial activity of clavulanate). Journal of Antimicrobial Chemotherapy, 2003; 52: 18–23.
7. White, R. A., Poupard, J., Pypstra, R. and Wynee. B. (Augmentin® (amoxicillin/clavulanate) in the treatment of community-acquired respiratory tract infection: a review of the continuing development of an innovative antimicrobial agent). Journal of Antimicrobial Chemotherapy, 2004; S1: i3–i20.
8. Glaxo Smith Klinegroup of companies. Augmentin data sheet, 2013.
9. Slocombe, B., Beale, A., Boon, R. J. et al. (Antibacterial activity in vitro and in vivo of amoxicillin in the presence of clavulanic acid). Postgraduate Medicine, Sept/Oct 1984; 29–49.

10. Cheesbrough. Medical Laboratory Manual for Tropical Countries. Volume II, Microbiology, 2005; 225-274.
11. Hi Media data sheet. Antibiotic Susceptibility Test, 2014.
12. United States Pharmacopeia. Antibiotic Microbial Assay; USP37-NF32: 2014.
13. Summary of Product Characteristics (SPC), published by electronic Medicines Compendium (eMC), https://www.medicines.org.uk/emc/medicine/19191.
14. Abdelhaleem. (Evaluation of antimicrobial resistance of urinary tract isolated Esherichia coli from Omdurman Teaching Hospital in Sudan). Journal of Bacteriology Research, 2013; 5(6): 76-77.