DETECTION OF ESBL, AMPC AND METALLO BETA- LACTAMASE MEDIATED RESISTANCE IN GRAM- NEGATIVE BACTERIA ISOLATED FROM WOMEN WITH GENITAL TRACT INFECTION[1]

Shilan S. Ahmad
Department of Laboratory, Raparin Pediatric Teaching Hospital, Erbil, Iraq
Fattma A. Ali
Department of Microbiology, College of Medicine,
Hawler Medical University, Erbil, Iraq

Abstract

This study was carried out during the period (March to July 2012). A total of two hundred and fifty high vaginal swabs were collected from (100 pregnant and 150 non- pregnant) women patients with genital tract infection at the age ranged between (18- 55) years, who attended the gynecology clinics and obstetrics department of Maternity Teaching Hospital in Erbil city. Vaginal swab samples were collected and direct examined for microscopic Gram stain examination and culture techniques. Isolated microorganisms were identified using microscopical, morphological, biochemical tests, analytic profile index system and also identification and sensitivity test were performed by Vitek 2 compact system. The results showed that positive vaginal cultures of Gram- negative bacteria isolates obtained from women patients were 73 isolates, which distributed among pregnant 20 (27.4%) and non- pregnant women 53 (72.6%) is not significant according to statistical analysis. All Gram negative bacterial (73) isolates were screened for their ability to produce extended spectrum β- lactamases enzymes by using double disk diffusion method. Out of 45 (61.6%) were found to be extended spectrum β- lactamases producers, which distributed among pregnant 9 (45%) and non- pregnant 36 (67.9%) but statistical analysis not significant. All Gram negative bacteria were screened for their ability to produce Ampicillin resistant gene (AmpC) β- lactamase enzyme by using Disk antagonism test. Out of (73) Gram negative bacteria isolates, 5 (6.8%) were found to be AmpC β- lactamase producers. All Gram negative

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bacteria were screened for their ability to produce metallo β- lactamase enzyme by using Imipenem- EDTA (Ethylene diamine tetra acetic acid) combined disc test. Out of (73) Gram negative bacteria isolates, 25 (34.2%) were found to be metallo β- lactamase producers, which distributed among pregnant 5 (25%) and non- pregnant 20 (37.7%). Among all Gram negative bacterial isolates were screened for their ability to produce (Extended spectrum β- lactamases, AmpC and metallo β- lactamase) enzymes and the results revealed that most of isolates produce more than one type of β- lactamase enzymes, for example all *Escherichia coli* isolates 30 (71.4%) were extended spectrum β- lactamases producers and 19 (45.2%) were metallo β- lactamase producers.

Keywords: ESBL, AmpC and Metallo β- lactamase, Gram- negative bacteria, Genital tract infection

Introduction

Vaginitis is an infectious inflammation of the vaginal mucosa, which some times involves the vulva. This inflammation often causes itching, burning, irritation, discharge and discomfort. It is one of the most common reasons for women to seek medical care (Egan and Lipsky, 1999).

Vaginitis, whether infectious or not, poses one of the most common problems in gynaecology, and is one of the main reasons leading the females to seek advice from a physician approximately 10 million office visits annually (Syed and Braverman, 2004).

Several publications have also reported an altered vaginal microflora being linked to an increased susceptibility to other vaginal microorganisms include facultative anaerobes, coliforms, true anaerobes, and non- bacterial microorganism such as *Candida albicans.* Many of them are commensal; they live within this ecosystem but do not harm to the host (Alim *et al*., 2009).

β- lactamases are enzymes produced by some bacteria that hydrolyze the β-lactam ring of β- lactam antibiotics (penicillins, ceohalosporins, monobactams, and carbapenems), is one of the most important mechanisms of microbial resistance to β- lactam antibiotics (Noyal *et al*., 2009). The hydrolyzed β-lactam drugs result in an inactive product when the ring is broken (Simoens *et al*., 2006).

One of the most important resistant mechanisms in Gram-negative bacteria against β- lactam antibiotics is induced by production of β- lactamase enzymes. Indeed, occurrence of point mutations in the sequence of the primary β- lactamase gene results in production of different enzymes. β- lactamase enzymes are classified into four main groups including A, B, C and D. According to this classification, broad- spectrum β- lactamases are

categorized among group A. The Gram-negative bacteria have rapidly expanded resistance to broad- spectrum β- lactam antibiotics. More than 200 types of extended- spectrum β- lactamases (ESBLs) have been found worldwide, most belonging to the Enterobacteriaceae family (Yazdi *et al*., 2012).

ESBL are bacterial enzymes that hydrolyse and confer resistance to modern cephalosporin antibiotics. They constitute the major mechanism of resistance to second, third and fourth generation cephalosporins for example: cefuroxime, cefotaxime, ceftriaxone and ceftazidime (Lavilla *et al*., 2008). Organisms often also possess resistance determinants to other antibiotic groups, such as aminoglycosides and fluoroquinolones, leaving an extremely limited range of effective agents (Weinbren and Borthwick, 2005).

The ESBL producing *E. coli* are difficult to treat due to their resistance to wide spectrum of antibiotics including the third generation cephalosporine. Factors often responsible for *Escherichia coli* resistance include R-factor on plasmids, resistance genes on the chromosomes, production of β- lactamase and extended spectrum β-lactamase enzymes (Aboderin *et al.* ,2009).

The prevalence of ESBL among clinical isolates varies among geographic areas with low rates of (3– 8%) in Sweden, Japan and Singapore to much higher prevalence rates reported from Portugal (34%), Latin America (30–60%), and Turkey 58% (Paterson and Bonomo, 2005).

AmpC β- lactamases are Cephalosporinases which are poorly inhibited by Clavunic acid. They are differentiated from other ESBLs by their ability to hydrolyze Cephalosporins as well as other extended spectrum Cephalosporins (Manchanda and Singh, 2003).

Several bacterial species *Enterobacter* spp., *Citrobacter freundii*, *Pseudomonas* spp., and *Serratia marcescens* have inducible chromosomally encoded AmpC cephalosporinase. Either inducibility or stable overproduction of this enzyme, resulting from mutation (Drieux *et al*., 2008).

Many clinical laboratories currently test *Escherichia coli* and *Klebsiella* spp. for production of ESBLs but do not attempt to detect plasmid mediated AmpC β- lactamases (also known as imported, transmissible, foreign or mobile AmpC β- lactamases). These enzymes are typically associated with multiple antibiotic resistances (Black *et al*., 2005). It is important to know the occurrence of ESBL and AmpC producing strains as well as their antibiotic susceptibilities to newer agents to guide empirical therapy for various infections (Taneja *et al*., 2008).

ESBLs and AmpC β- lactamases are of increasing clinical concern. ESBLs are most commonly produced by *Klebsiella* spp. and *E. coli* but may also occur in other Gram- negative bacteria. They are typically plasmid mediated, clavulanate susceptible enzymes that hydrolyze penicillins,

expanded- spectrum cephalosporins (cefotaxime, ceftriaxone, ceftazidime, cefepime and others) and aztreonam (Moland *et al.,* 2002).

Carbapenems have been the most successful β- lactam antibiotics used in the treatment of infections caused by β- lactam resistant Gram-negative bacteria. The clinical utility of these antimicrobials is under threat with the emergence of carbapenemases, particularly the class B metallo β-lactamases (MBLs). MBLs can hydrolyze most β- lactams except for monobactams and confer a broad- spectrum β- lactam resistance to the bacterial host, which is not reversible by conventional therapeutic β-lactamase inhibitors. The prevalence of MBLs has been increasing worldwide, notably among *Pseudomonas aeruginosa* and lately, amongst other Gram- negative bacteria as well (Walsh *et al*., 2005).

MBLs producing Gram- negative bacteria an increasing public health problem worldwide because of their resistance to all β- lactams except aztreonam (Cornaglia *et al*., 2007). MBL genes are either carried transferable plasmids or are part of the bacterial chromosome (Walsh *et al*., 2005).

Materials and Methods

Samples collection: High vaginal swabs were collected from two hundred and fifty (250) women patients with vaginal symptoms who attended the gynecology clinics and obstetrics department of Maternity Teaching Hospital in Erbil city during the period from March to July 2012. All vaginal swabs were taken from married women patients, of these 100 swabs from pregnant and 150 were from non-pregnant women. The age of these patients ranged between (18- 55) years.

High vaginal swabs were taken from women patients suffering with abnormal vaginal discharge, itching, burning and lower abdominal pain. The samples were taken from each women patient (by doctors) using sterile swabs stick and speculum. Vaginal swab for each patient were transported to the laboratory by inoculating the swab into a sterile tube containing 3 ml of normal saline. The samples were examined by staining with Gram stain was performed.

Isolation of microorganisms: For isolation of microorganisms, the specimen of vaginal swab was directly inoculated on culture media: Blood agar, MacConkey agar at 37˚C for 24-48 (Oladele *et al.,* 2011; Razzak *et al.,* 2011).

Identification of microorganisms: Pure colonies of isolated microorganisms were identified using morphological, biochemical tests including API system (Forbes *et al.,* 2002). Species identification and antibiograms for pathogens were performed using Vitek 2 compact system (Nagaraja, 2008)**.**

Antimicrobial susceptibility testing: The susceptibility test was performed on 73 isolates belonging to different species of Gram negative bacteria by Vitek 2 compact system to different antibiotics (31) are represented in table (3), and also by Disc diffusion method, also known as the Kirby- Bauer method was carried out according to the Clinical and Laboratory Standard Institute guidelines (CLSI), formerly the National Committee for Clinical Laboratory Standards (NCCLS) (Wayne, 2005) were performed against 30 antibiotics.

Detection of β- lactamase in Gram negative bacteria

A total of 73 samples isolates of Gram negative bacteria were screened for different type of β- lactamase enzyme which were responsible for resistant mechanisms in Gram negative bacteria.

Detection of extend spectrum β- lactamase (ESBL)

Confirmatory test for detection of ESBL by double disc diffusion test: Extend spectrum β- lactamases (ESBLs) are plasmid– mediated enzymes produced by a number of Gram negative bacteria (Collins *et al.*, 2004). A double disc diffusion test was performed with amoxicillin-clavulanic acid surrounded by aztreonam and third generation cephalosporin discs cefotaxime and ceftazidime (Freitas *et al.*, 2003).

Detection of AmpC β- lactamase

Confirmatory test for detection of AmpC enzymes by disk antagonism test: Tested isolates with a turbidity equivalent to that of 0.5 McFarland standards was spread over a Muller Hinton agar plate. Cefotaxime (30µg/ml) and Cefoxitin (30µg/ml) disks were placed 20 mm apart from center to center, after overnight incubation an isolates showing blunting of the cefotaxime zone of inhibition adjacent to the cefoxitin disk or reduced susceptibility to each of them were screened as positive for AmpC β-lactamase production (Smatha and Parveen, 2011).

Detection of metallo β- lactamase:

Imipenem (IMP) - EDTA combined disc test: The IMP- EDTA combined disk test was performed by the test organisms were inoculated on to plates with Muller Hinton agar as recommended by the CLSI **(**Institute, 2006). Two (10µg/ml) imipenem disks were placed on the plate, and appropriate amounts of 10µl of EDTA solution were added to one of them to obtain the desired concentration (750µg/ml). The inhibition zones of the imipenem and imipenem- EDTA disks were compared after 18- 24 hours of incubation at 37˚C. In the combined disc test, if the increase in inhibition zone with the Imipenem and EDTA disc was $\geq$ 7 mm than the Imipenem disc

alone, it was considered as Metallo β- lactamase positive (Behera *et al.*, 2008).

Solution of a 0.5 M (Molarity) EDTA was prepared by dissolving 186.1 gm of EDTA. 2H2O in 1000 ml of distilled water and its pH was adjusted to 8.0 by using NaOH and sterilized by autoclaving (Yong *et al.*, 2002).

Results and discussion

A total of two hundred and fifty (250) high vaginal swabs were collected from women patients attending Maternity Teaching Hospital in Erbil city suspected of having vaginitis (We exclude the patients who are unmarried). The results of this study indicated that 73 (38.2%) of Gram-negative bacteria with vaginitis detected in pregnant and non- pregnant women with symptoms as illustrated in table (1). The statistical analysis showed no significant differences of infection among non- pregnant and pregnant. Statistical difference were determined by Chi- square (X^2) test. Probability value (P-value) less than (< 0.05) was considered as statistically significant (*), while P-value more than (> 0.05) was considered as statistically not significant.

This result in agreement with those dictated by Razzak *et al*., (2011) in Babylon (Iraq) showed that (44.8%) samples gave positive culture for Gram- negative bacteria, and agree with Al- Muk and Hansony (2001) from Basrah (Iraq), they reported in pregnant women the rate of Gram- negative bacteria were (13.5%), and agree with Saini *et al*., (2003) from India, he reported the Gram- negative bacteria (47.4%).

The results also showed that the percentage of positive culture in pregnant women 20 (30.3%) were higher than non- pregnant women 53 (42.2%), but statistically not significant difference. Our results seem to agree with finding by Jarjees (2006) from Erbil (Iraq) who reported in pregnant (71%) and in non- pregnant (67%). The high incidence of infection in pregnant women could be attributed to hormonal changes (Greeenwood *et al*., 2002).

The presence of this bacteria in large percent in urinary tract and bacterial vaginosis might be attributed to the fact that this bacteria is part of the normal fecal flora and different virulence factors contributing to their pathogenicity and the difference in the result might be attributed to the number of sample taken and the difference in the time (year) of the study.

The most common Gram negative bacteria isolated from the vaginal women with vaginitis was *Escherichia coli* 42 (57.5%), also the frequency of *Escherichia coli* in non- pregnant 30 (56.6%) higher compared to pregnant 12 (60%) are showed in table (2). These results were agreement with those

reported in our country such as by Jarjees (2006) from Erbil (Iraq) in non-pregnant (53.2%) and in pregnant (48.6%).

The low frequency of infection noticed by *Pseudomonas aeruginosa* was 2 (2.7%) and *Pseudomonas luteola* was 1 (1.4%). Similar findings were obtained by Al- Jammaly and Abdulla (2008) from Mosul (Iraq) (1.9%) and Mumtaz *et al*., (2008) from Pakistan (1.8%), they reported that the incidence of infection with *Pseudomonas aeruginosa*.

In the present study the antimicrobial susceptibility test done by Vitek 2 compact system, The result showed that in table (3) among Gram-negative bacteria the most effective antibiotics that have low percentage of resistance were Meropenem (0%) but for Imipenem was 5 (6.8%) and Amikacin was 9 (12.3%) when used for all tested Gram- negative bacteria. While the isolates showed high percentage of resistance to Ampicillin 65 (89%), followed by Amoxicillin/ clavunic acid 41 (56.2%), Aztreonam and Clindamycin 39 (53.4%) for each.

Similar finding have been obtained by Jarjees (2006) from Erbil (Iraq) who reported that the percentage of resistance to Imipenen (13%) and Amikacin (10%), while the high resistance to Penicillin (89%), Amipicillin (79.5%) and Tetracycline (72.5%) among gram positive and negative bacterial isolated from genitourinary tract infection. Oladele *et al.,* (2011) from Nigeria reported the incidence of antibiotic resistance among bacterial isolated from vaginitis were Amoxicillin/ clavunic acid (60%), Amipicillin (60%), Tetracycline (40%).

Extended spectrum β- lactamases (ESBLs) are usually inhibited by β-lactamase inhibitors, such as clavulanic acid, sulbactam or tazobactam. Therefore, use of β- lactam/ β- lactamase inhibitor combinations has been considered for the treatment of infections due to ESBL- producing organisms. In addition, increased use of carbapenems to treat ESBL-producing organisms has been associated with the emergence of carbapenem- resistant organisms **(**Rupp and Fey, 2003).

The result represent ESBL production occurred in 45 (61.6%) out of (73) Gram- negative bacteria isolates as seen in table (4) an figure (1), with highest incidence in *Enterobacter aerogenes*, *Serratia fonticola*, *Raoultella ornithinolytica*, *Pantoea agglomerans* and *Sphingomonas paucimobilis* (100%) for each, followed by *E. coli* 30 (71.4%), *Klebsiella pneumoniae* 7 (50%), *Proteus mirabilis* 2 (40%). When comparing the result to other study we notice similar finding have been obtained by Al- Haidari (2010) from Erbil (Iraq) who found that (76.3%) of Gram- negative bacteria isolates were ESBL producer and showed that ESBL production ranged among *E. coli* (77.1%), *Proteus mirabilis* (42.9%) and *Enterobacter* spp. (100%). Al-Nammi (2001) in Baghdad (Iraq), who reported that (72.6%) of *E. coli* isolates gave positive ESBL.

But different results have been obtained by Jarjees (2006) from Erbil (Iraq) who reported that ESBL production by BV ranged among *E. coli* (86.07%), *Proteus mirabilis* (55.55%), *Klebsiella pneumoniae* (80%) and *Enterobacter aerogenes* (60%). Al- Zarouni *et al.*, (2008) in Unit Arab Emirates, who found that the ESBL production in *E. coli* (39%), *Klebsiella pneumoniae* (42%). Yasushisa (1994) from Japan who found that the production of ESBL enzymes were (84.7%) in *E. coli* and (65.4%) in *Klebsiella pneumoniae*.

Many clinical microbiological laboratories still face significant problems with ESBL screening and identification as ESBL pathogens can present with variations in the in vitro pattern of resistance to β- lactam agents. Proficiency-testing studies performed by the World Health Organization and the Centers for Disease Control have raised concerns about the current ability of many clinical laboratories to detect ESBL-producing microorganisms (Hageman *et al*., 2003).

On the other hand the results in the present study also showed that out of (73) Gram- negative bacteria only 5 (6.8%) isolates were produce AmpC β- lactamase as shown in figure (2), showed that AmpC β- lactamase production ranged among *Pseudomonas luteola* and *Ewingella americana* 1 (100%) each, followed by *Pseudomonas aeruginosa* 1 (50%) and *Proteus mirabilis* 2 (40%). Similar results were reported by Sanguinetti *et al*., (2003) from Italy reported (11.6%) AmpC β- lactamase positive among Gram-negative bacteria isolates.

Other result reported by Samatha and Praveen (2011) from India who showed that among gram negative bacteria (24.6%) screen positive to AmpC, which include *Pseudomonas* spp. (33.3%) and *Proteus* spp. (16.7%) were screen positive to AmpC.

Among the β- lactamases the production of ESBLs and AmpC β-lactamases are the most common mechanisms for resistance to β- lactam antibiotics in Gram negative bacteria (Taneja *et al*., 2008).

Carbapenems have a broad spectrum of antibacterial activity and these are resistant to hydrolysis by most β- lactamases including ESBLs and AmpC β-lactamases. These are often used as a last resort in infections due to multi drug resistant Gram- negative bacilli (Noyal *et al.*, 2009).

Furthermore the results in the present study showed that out of (73) Gram negative bacteria tested 25 (34.2%) isolates were produce metallo β-lactamase as seen in figure (3), They were distributed among *E. coli* 19 (45.2%), *Klebsiella pneumoniae* 5 (35.7%) and *Sphingomonas paucimobilis* 1 (100%). Similar results were reported by Enwuru *et al.*, (2011) from Nigeria reported that among Gram- negative bacterial strains tested (23%) were confirmed to be MBL producer, in this *E. coli* (50%) and *Klebsiella spp.* (36%).

MBLs producing Gram-negative bacteria are an increasing public health problem world wide because of their resistance to all β- lactam except Aztreonam. MBL genes are typically carried on transferable plasmids or are part of the bacterial chromosome. This enzyme which have been detected primarily in *Pseudomonas aeruginosa* but were also found in other Gram-negative bacteria, including nonfermenters and members of the family Enterobacteriaceae (Valenza *et al.,* 2010).

The awareness of the existence of MBL initializes indication for the need for proper use of antibiotics and spread of multi drug resistance bacterial strains within these hospital and communities.

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Table (1): Distribution of Gram- positive and Gram- negative bacteria in relation to pregnant and non- pregnant women with vaginitis

Bacterial vaginosis	Non- pregnant		Pregnant		Total	
	No.	%	No.	%	No.	%
Gram- positive bacteria	72	57.6 %	46	69.7 %	118	61.8 %
Gram- negative bacteria	53	42.4 %	20	30.3 %	73	38.2 %
Total	125	65.4 %	66	34.6 %	191	100 %
Chi- square (x^2)	2.68 N.S.					

Note: N.S. = No Significant

Table (3): Distribution of Gram- negative bacteria in vaginitis in relation to pregnant and non- pregnant women

Isolated Gram- negative bacteria	Non- pregnant		Pregnant		Total	
	No.	%	No.	%	No.	%
Escherichia coli	30	56.6 %	12	60 %	42	57.5 %
Klebsiella pneumoniae	12	22 .6%	2	10 %	14	19.1 %
Proteus mirabilis	3	5.6 %	2	10 %	5	6.8 %
Pseudomonas aeruginosa	2	3.8 %	0	0 %	2	2.7 %
Pseudomonas luteola	1	1.9 %	0	0 %	1	1.4 %
Serratia fonticola	1	1.9 %	1	5 %	2	2.7 %
Serratia plymuthica	1	1.9 %	0	0 %	1	1.4 %
Enterobacter aerogenes	0	0 %	1	5 %	1	1.4 %
Acinetobacter lwoffii	1	1.9 %	0	0 %	1	1.4 %
Raoultella ornithinolytica	1	1.9 %	0	0 %	1	1.4 %
Pantoea agglomerans	0	0 %	1	5 %	1	1.4 %
Sphingomonas paucimobilis	0	0 %	1	5 %	1	1.4 %
Ewingella americana	1	1.9 %	0	0 %	1	1.4 %
Total	53	72.6 %	20	27.4 %	73	100 %

Table (4): The number and percentage of antibiotics resistance in Gram negative bacteria

Total No. of isolated bacteria	1 AMP	2 AX	3 AMC	4 AK	5 ATZ	6 CIP	7 CD	8 CX	9 CRO	10 CTX	11 CAZ	12 CEFE	13 COT	14 CEFL	15 CEFP
Escherichia coli (42)	40 95.2%	*	26 61.9 %	7 16.7 %	25 59.5%	14 33.3%	20 47.6%	5 11.9%	20 47.6%	21 50%	23 54.8%	15 35.7%	8 19%	2 4.8%	9 21.4 %
Klebsiella pneumoniae (14)	14 100%	*	8 57.1 %	1 7.1%	7 50%	5 35.7%	6 42.9%	1 7.1%	6 42.9%	7 50%	6 42.9%	3 21.4%	1 7.1%	2 14.3%	2 14.3 %
Proteus mirabilis (5)	5 100%	*	3 60%	0	0	2 40%	5 100%	0	*	2 40%	2 40%	*	*	2 40%	*
Pseudomonas aeruginosa (2)	2 100%	*	2 100%	0	0	0	2 100%	2 100%	2 100%	2 100%	0	0	*	1 50%	1 50%
Pseudomonas luteola (1)	1 1005	*	0	0	1 100%	0	1 100%	1 100%	0	0	0	0	*	0	0
Serratia fonticola (2)	1 50%	0	0	0	2 100%	1 50%	1 50%	0	1 50%	2 100%	0	0	*	*	0
Serratia plymuthica (1)	0	0	0	0	0	0	0	0	0	0	1 100%	0	*	*	0
Enterobacter aerogenes (1)	1 100%	*	1 100%	0	1 100%	0	1 100%	1 100%	1 100%	1 100%	0	0	*	1 100%	*
Acinetobacter lwoffii (1)	0	*	0	0	0	0	0	0	0	0	0	0	*	1 100%	0
Raoultella ornithinolytica (1)	1 100%	*	0	0	1 100%	1 100%	1 100%	1 100%	1 100%	1 100%	0	0	*	*	*
Pantoea agglomerans (1)	0	*	0	0	1 100%	0	0	0	*	0	0	0	*	*	*
Sphingomonas paucimobilis (1)	0	*	0	1 100%	1 100%	0	1 100%	1 100%	0	0	0	0	*	*	*
Ewingella americana (1)	0	1 100%	1 100%	0	0	0	1 100%	0	0	0	0	1 100%	*	*	*
Total No. 73 & % in total of antibiotic used	65 89%	1 0.25 %	41 56.2 %	9 12.3 %	39 53.4%	23 31.5%	39 53.4%	12 16.4%	31 46.3%	36 49.3	32 43.8%	19 28%	9 16.1 %	9 13.6%	12 19%

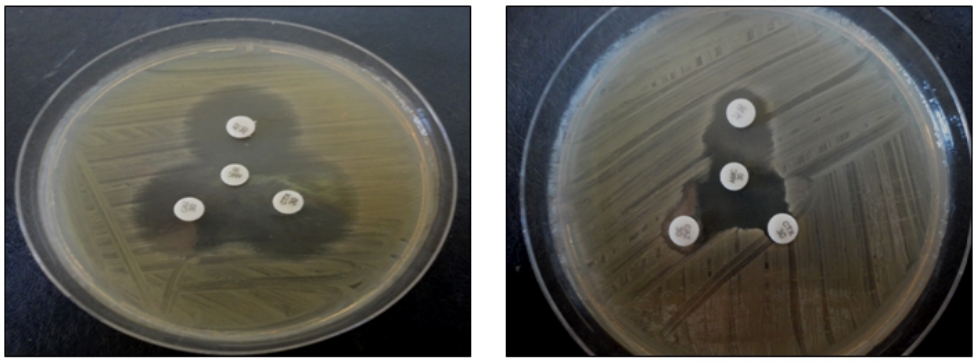
* These antibiotics were not used (not done); AMP (Ampicillin), AX (Amoxicillin), AMC (Amoxicillin clavunic acid), AK (Amikacin), ATZ (Aztreonam), CIP (Ciprofloxacin), CD (Clindamycin), CX (Cefoxitin), CRO (Ceftriaxone), CTX (Cefotaxime), CAZ (Ceftazidime), CEFE (Cefepime), COT (Co- trimidazole), CEFL (Cefalotin), CEFP (Cefpodoxime).

Total No. of isolated bacteria	16 CEFU	17 CEFA	18 E	19 GEN	20 IMP	21 NIF	22 NOR	23 NA	24 PIP	25 VA	26 RA	27 TOB	28 TE	29 MER	30 TCC	31 TRIM
Escherichia coli (42)	10 23.8%	20 47.6%	16 38.1%	13 31%	1 2.3%	4 9.5%	*	6 14.3%	9 21.4%	16 38.1 %	16 38.1%	6 14.3%	15 35.7%	0	13 31%	16 38.1%
Klebsiella pneumoniae (14)	2 14.3%	4 28.9%	6 42.9%	4 28.9%	0	8 57.1%	*	2 14.3%	4 28.9%	6 42.9 %	6 42.9%	3 21.4%	5 35.7%	0	*	4 28.9
Proteus mirabilis (5)	2 40%	*	*	3 60%	2 40%	5 100%	2 40%	*	0	*	*	0	5 100%	0	*	5 100%
Pseudomonas aeruginosa (2)	1 50%	2 100%	*	0	0	2 100%	0	*	0	*	0	0	2 100%	*	*	2 100%
Pseudomonas luteola (1)	0	0	*	0	0	1 100%	0	*	0	*	0	0	0	*	*	1 100%
Serratia fonticola (2)	*	1 50%	*	0	0	0	0	*	0	*	*	1 50%	0	*	*	0
Serratia plymuthica (1)	*	1 100%	*	0	0	0	0	*	0	*	*	0	0	*	*	1 100%
Enterobacter aerogenes (1)	*	1 100%	*	1 100%	0	0	0	*	0	*	*	1 100%	0	0	*	1 100%
Acinetobacter lwoffii (1)	0	0	*	0	0	1 100%	0	*	0	*	*	0	0	0	*	0
Raoultella ornithinolytica (1)	1 100%	1 100%	*	1 100%	1 100%	1 100%	1 100%	*	0	*	*	1 100%	1 100%	*	*	1 100%
Pantoea agglomerans (1)	0	0	*	0	0	0	0	*	0	*	*	*	0	*	*	0
Sphingomonas paucimobilis (1)	0	0	*	0	0	0	0	*	0	*	*	*	0	*	*	0
Ewingella americana (1)	*	*	*	1 100%	1 100%	1 100%	1 100%	*	0	*	*	1 100%	1 100%	*	0	1 100%
Total No. 73 & % in total of antibiotic used	16 23.5%	30 44.8%	22 39.3%	23 31.5%	5 6.8%	23 31.5%	4 23.5%	8 14.3%	13 17.8%	22 39.3 %	22 37.3%	13 18.3%	29 39.7%	0 0%	13 30.2%	32 43.8%

CEFU (Cefuroxime), CEFA (Cefazolin), E (Erythromycin), GEN(Gentamicin), IMP (Imipenem), NIF (Nitrofurantoin), NOR (Norfloxacin), NA (Nalidixic acid), PIP (Piperacillin), VA (Vancomycin), TOB (Tobromycin), RA (Rifampicin), TE (Tetracycline), TRIM (Trimethoprim), TCC (Ticarcillin clavunic acid), MER (Meropenem),

Table (5): Frequency of ESBL and AmpC β- lactamase and metallo β-lactamase producing Gram- negative bacteria

Gram- negative bacteria isolated	Total No. of isolated	No. & % of isolated with ESBL		No. & % of isolated with AmpC β- lactamase		No. & % of isolated with Metallo β-lactamase	
		Positive	Negative	Positive	Negative	Positive	Negative
Escherichia coli	42	30 (71.4%)	12 (28.6%)	0 (0 %)	42 (100%)	19 (45.2%)	23 (54.8%)
Klebsiella pneumoniae	14	7 (50%)	7 (50%)	0 (0 %)	14 (100%)	5 (35.7%)	9 (64.3%)
Proteus mirabilis	5	2 (40%)	3 (60%)	2 (40 %)	3 (60%)	0 (0 %)	5 (100%)
Pseudomonas aeruginosa	2	0 (0 %)	2 (100%)	1 (50%)	1 (50%)	0 (0 %)	2 (100%)
Pseudomonas luteola	1	0 (0 %)	1 (100%)	1 (100%)	0 (0 %)	0 (0 %)	1 (100%)
Serratia fonticola	2	2 (100%)	0 (0 %)	0 (0%)	2 (100%)	0 (0 %)	2 (100%)
Serratia plymuthica	1	0 (0 %)	1 (100%)	0 (0 %)	1 (100%)	0 (0 %)	1 (100%)
Enterobacter aerogenes	1	1 (100%)	0 (0 %)	0 (0 %)	1 (100%)	0 (0 %)	1 (100%)
Acinetobacter lwoffii	1	0 (0 %)	1 (100%)	0 (0 %)	1 (100%)	0 (0 %)	1 (100%)
Raoultella ornithinolytica	1	1 (100%)	0 (0 %)	0 (0 %)	1 (100%)	0 (0 %)	1 (100%)
Pantoea agglomerans	1	1 (100%)	0 (0 %)	0 (0%)	1 (100%)	0 (0 %)	1 (100%)
Sphingomonas paucimobilis	1	1 (100%)	0 (0 %)	0 (0 %)	1 (100%)	1 (100%)	0 (0 %)
Ewingella americana	1	0 (0 %)	1 (100%)	1 (100%)	0 (0 %)	0 (0 %)	1 (100%)
Total	73	45 (61.6%)	28 (38.4%)	5 (6.8%)	68 (93.2%)	25 (34.2%)	48 (65.8%)

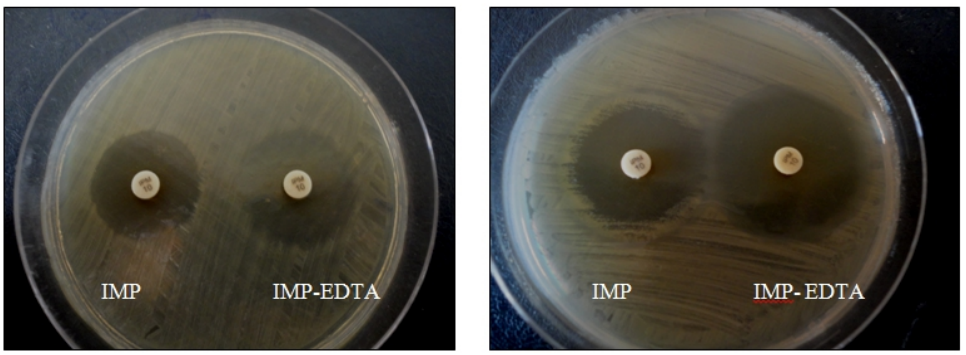


A B

Figure (1): Double- disk diffusion test used for the detection of ESBL production in Gram negative bacteria; (A) ESBL negative, (B) ESBL positive

A B

Figure (2): Disk antagonism test used for the detection of AmpC β- lactamase production in Gram negative bacteria; (A) AmpC β- lactamase negative (Absence of blunting indicates negative), (B) AmpC β- lactamase positive (Blunting of the cefotaxime disc adjacent to the cefoxitin disk, positive)



A B

Figure (3): Imipenem (IMP)- EDTA combined disc test used for the detection of metallo β- lactamase production in Gram negative bacteria; (A) Metallo β- lactamase negative, (B) Metallo β- lactamase positive (IMP- EDTA increase clear zone of inhibition).