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# PREVALENCE OF MULTIDRUG-RESISTANT (MDR) AMONG CLINICAL BACTERIAL ISOLATES IN EL-GHARBIA GOVERNORATE AND EFFICIENCY OF ANTIBIOTIC COMBINATIONS

**Mervat Aly Abo-State[1*], Soheir Saad Abd El-Salam[2] and Rania Assi[2]**

[1]Radiation Microbiology Department, National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA), Nasr CITY, Cairo, Egypt.

[2]Microbiology Department, Faculty of Science, Benha University, Egypt.

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***Corresponding Author**
**Mervat Aly Abo-State**
Radiation Microbiology Department, National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA), Nasr CITY, Cairo, Egypt.

## ABSTRACT

Prevalence of multidrug-resistance (MDR) bacteria represents a great problem worldwide. Survey to determine the susceptibility profile of clinical bacterial pathogenic strains to the most commonly prescribed antibiotics by physicians in Egyptian hospitals have been determined between 20/5/2013 to 15/9/2014. Out of 200 bacterial isolates, 144(72%) were Gram-negative bacilli (GNB), 54(27%) Gram-positive cocci and two (1%) were *Candida* spp. The most frequent pathogens were *Klebsiella* spp. (42%) followed *Staph. aureus* (16%) and *E.coli* (15.5%). The most resistant isolates were *Pseudomonas* spp. (79 %). The most efficient antibiotic was amikacin (AK) (48.5%), while most bacterial isolates were resistant to spectinomycin (SPT) (93%). The results showed that aminoglycoside antibiotics were the most efficient agents against the clinical bacterial isolates. And from the beginning to the end of the collection period, it was found that MDR bacterial isolates 186 isolates out of 200 (93%), these MDR involved 59 XDR isolates which represented 29.5%. The sensitive isolates were found to be 14 isolates (7%) from all the isolated strains. The results of $MIC_{50}$ proved that amikacin (AK) having the lowest MIC against most MDR strains tested when compared with the two other antibiotics (aztreonam (ATM) and meropenem (MERO)). This results confirmed the previous results of the present study that AK was the most effective antibiotics. The percentage of growth inhibition of double, triple and four antibiotic

combinations revealed that inhibition exceeded 95% for most combinations.

**KEYWARDS:** Antibiotic resistance, Pathogenic bacteria, susceptibility, MDR, XDR, antibiotic combinations.

## INTRODUCTION

Bacterial infections are the major cause of morbidity and mortality worldwide.[1] Antibiotic resistance poses an increasingly grave threat to the public health. Of pressing concern, rapid spread of carbapenem-resistance among multidrug-resistant (MDR) Gram-negative rods (GNR) is associated with few treatment options and high mortality rates.[2]

Urinary tract infections (UTIs) are one of the major causes of prescribing and antibiotic consumption. In order to use the best antibiotic treatment for their patients, reliable and recent data about epidemiology and antibiotic resistance profile of uropathogenic bacteria must be available for clinicians. Therefore regular monitoring in each country is required.[3] Among the uropathogenic bacteria, *Escherichia coli* is predominant in both community and nosocomial UTI. However, the diversity of uropathogens is known to vary regionally.[4] The second most widespread type of infections in the human body is UTI and it has an effect on millions of people annually. The Existence of resistance even to most potent antibiotics is leading to increasing the rate of antibiotics consumption to treat infections.[5,6]

Recent reports by the U.S. Centers for Disease Control and Prevention[7] and the World Health Organization[8] describe this ever-worsening antibiotic resistance problem, the presence of carbapenem-resistant Gram-negative rods (GNRs) makes the human medicine enter into a “post-antibiotic era”. Rapid resistance prevalence of carbapenem *in Pseudomonas aeruginosa* (PA), *Klebsiella pneumoniae* (KP) and *Acinetobacter baumannii* (AB) is a serious problem because the antibiotic agents are currently in need of the development.[9]

MDR Gram-positive bacteria are less prevalent than MDR Gram-negative ones.[10] Gram-positive bacteria, specially Gram-positive cocci of the genera *Enterococcus*, *Staphylococcus* and *Streptococcus*, have serious pathogenic species causing critical infections and associated with morbidity and mortality.[11-16]

Monotherapy to treat these infections is no longer enough because of the high in the presence of other MDR bacteria, especially Gram-negative bacteria so the use of combination treatments is necessary.[17] As a result of the spread of multi-extended- or pan-resistant

bacteria has extremely increased throughout the years, the therapy of some infectious diseases is currently so difficult.[18] So, alternative approaches are seriously needed to controlling the infections of bacteria. The combination of two or even more antimicrobial drugs during a treatment system is the only approach to fighting MDR infections.[19]

The aim of the present study was to determine the prevalence of MDR and eXDR bacterial strains among patients of El-Gharbia Governorate, Egypt, and determination of the most effective antibiotics single or in combination against these strains.

## MATERIALS AND METHODS

### *Sampling sites*

The clinical samples were collected from three laboratories of the overall clinical of Tanta University Hospitals, Microbiology Department of Medicine of Tanta University, Tanta Tumors Center (laboratories of central health) and (laboratory of environmental balance) in El-Gharbia Governorate.

### *Isolation of clinical human bacterial pathogens*

Samples were collected during the period between 20/5/2013 and 15/9/2014 according to.[20] The samples were inoculated on Blood agar, MacConkey and Cystine lactose electrolyte deficient (CLED) agar plates. On the surface of each medium, the single isolate was selected from each sample using a standard calibrated loop.

### *Identification of the bacterial isolates*

Bacterial isolates resulted from culturing of the samples were identified based on the standard laboratory procedures involving morphological characteristics, Gram stain, rapid tests (catalase, oxidase, coagulase) and biochemical tests(indol, citrate, triple sugar iron, urease, oxidation, fermentation and hemolysin production).[20-21]

### *Antibiotic discs*

In the present study, 20 antibiotic discs have been used. These antibiotics were purchased from Bioanalyse LTD Tibbi Malzemeler San. ve Tic. Ltd. Sti Ankara/Turkey. The antibiotic discs were Penicillin G (P, 10 µg); Amoxicillin (AX, 25 µg); Amoxicillin/Clavonic acid(AMC,20/10 µg); Aztreonam (ATM,10 µg); Piperacillin (PRL,100 µg); Cefaclor (CEC,30 µg); Cephradine (CE,30 µg); Cefatoxime (CTX,30 µg); Cefepime (FEP, 30µg); Ciprofloxacine (CIP, 5 µg); Ofloxacin (OFX,5 µg); Pefloxacin (PEF,5 µg); Amikacin (AK,30

µg); Streptomycin (S, 10 µg); Sparfloxacin (SPX,5 µg); Spectinomycin (SPT, 10 µg); Gentamicin (CN,10 µg); Norfloxacin (NOR,10 µg); Tobramycin (TOB,10 µg) and Trimethoprim (TMP,5 µg).

***Antimicrobial susceptibility tests***

Antimicrobial susceptibility patterns were determined according to Clinical Laboratory Standard Institute[22] recommended modified Kirby-Bauer disc diffusion method on Mueller-Hinton agar plates.[23] A loopful of each clinical isolates was inoculated into 3.0 ml of L.B broth medium[24] and adjusted to $1\times10^3$ CFU/ml using Mc Farland Standards. About 0.1 ml of each isolate was inoculated and uniformly spread on the surface of Mueller- Hinton agar plates and the antibiotic discs were placed on the surface using sterile forceps under aseptic condition.[6] All plates were incubated up-right at 37°C for 24h. The inhibition zone diameter (mm) around each antibiotic disc has been determined. Two replicates were used for each antibiotic and each isolate. Those isolates which showed resistance to at least one antibiotic in three or more antimicrobial classes were considered MDR.[25]

***MIC determination***

Antimicrobial activity in terms of minimum inhibitory concentration (MIC) was determined[26] using L.B broth dilution method. Twelve MDR pathogenic isolates represent gram positive and gram negative bacterial isolates with MDR were selected to determine their MIC. The selected isolates were inoculated in L.B broth medium and incubated in shaking incubator (150 rpm) at 37°C for 24h. Four active ingredients (intravenous powder antibiotics) were aztreonam (ATM) product of Sanofi-Aventis, Paris, France; meropenem (MERO) product of ACS Dobfar, SPA, Italy for AstraZeneca UK Limited, Macclesfield, Cheshire, SK10 2NA,United Kingdom; amikacin (AK) from Sunny Pharmaceutical of Amoun Pharmaceutical Co. El-Obour City, Cairo, Egypt; and tigecycline (TGC) product of Patheon Italia, SPA, Monza(MB), Italy. The four antibiotics were purchased from their respective manufacturers. The stock solution of (50000 µg/ml) has been prepared. Twenty ml of sterilized L.B broth medium in 100 ml conical flask were supplemented with double fold dilution of antibiotic concentrations (0, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024 µg/ml) for each isolate in duplicates. The flasks were inoculated with 2 ml (~ $1\times 10^5$ CFU/ml) and incubated at 37°C for 18h in shaking incubator (150 rpm). The positive control was the bacterial strain in L.B broth without any antibiotic, while negative control was L.B broth medium without any inoculums of bacterial strains. The optical density (OD) was determined

spectrophotometrically at 600nm (spectrophotometer LW-V-200RS, Germany).

Percentage of growth inhibition= ((OD control-OD antibiotic)/OD control) × 100 where $MIC_{50}$ means the lowest concentration of antibiotic which resulted in 50% growth inhibition.[27]

***Determination of antibiotic combination efficacy***

Determination of the antibacterial activity of eight different antibiotic combinations has been conducted to six bacterial isolates with MDR as described by.[26] Each of the six selected MDR bacterial clinical isolates was inoculated in L.B broth medium and incubated in shaking incubator (150 rpm) for 24h at 37°C. Stock solutions of 40000 of AK, ATM, MERO and a stock solution 50000 of TGC have been prepared. The eight antibiotic combinations were ATM/MERO; ATM/AK; MERO/AK; ATM/ MERO/AK; ATM/MERO/TGC; ATM/AK/TGC; MERO/AK/TGC and ATM/MERO/AK/TGC. All experiments were carried out in duplicates. Twenty ml of sterilized L.B broth medium in 100ml conical flasks were supplemented with a double of its resistance breakpoint concentration that determined according to $MIC_{50}$ (minimum inhibitory concentration) values that were determined except tigecycline (TGC) that was used with concentration of 32 µg/ml for all the 6 clinical isolates. The flasks were inoculated with 2 ml (~ $1\times 10^5$ CFU/ml) and incubated in a shaking incubator (150 rpm) at 37°C for 18h. Positive and negative controls have been used and OD was determined at 600nm spectrophotometerically. The percentage of growth inhibition was also calculated.

## RESULTS

A total of 200 bacterial isolates isolated from patients hospitalized at El-Gharbia Governorate during the period between 20/5 / 2013 to 15/9/2014 were included in this study. This period was divided into five collection periods. The distribution of the samples were; urine (25%), endotracheal tube (ETT) (21%), wound (16.5%), each of throat swab and sputum (13.5%), blood (3%), stool (3%), pus (2%), food (1.5%) and Standardized strains from microbiology department from medicine of Tanta university (1%).

The frequency of bacterial strains isolated from different clinical samples between 20/5/2013 to 15/9/2014 was indicated in Table 1. As, *Klebsiella* spp. were represented 42% from the total isolates, while *Staph. aureus* and *E.coli* were 16% and 15.5% respectively. *Staph. epidermidis*, *Pseudomonas* spp., *Proteus* spp., *Streptococci*, *Candida* spp. 8%, 7.5%, 6%, 3%,

1% respectively. Each of *Salmonella* sp. and *Citrobacter* sp. was represented 0.5% as shown in Table 1.

**Table 1: Frequency of bacterial strains isolated from different clinical samples between 20/5/2013 to 15/9/2014.**

| Clinical isolates | No. of isolates | | | | | | |
|---|---|---|---|---|---|---|---|
| | Spring 20/5/2013 to 11/6/2013 | Autumn 30/10/2013 to 19/12/2013 | Winter 23/12/2013 to 10/3/2014 | Spring 22/3/2014 to 2/4/2014 | Summer 30/8/2014 to 15/9/2014 | Total | % |
| ***Klebsiella* spp.** | 9 | 31 | 18 | 4 | 22 | 84 | 42 |
| ***Pseudomonas* spp.** | 3 | 2 | 5 | 3 | 2 | 15 | 7.5 |
| ***E.coli*** | 5 | 6 | 9 | 6 | 5 | 31 | 15.5 |
| ***Staph. aureus*** | 4 | 7 | 6 | 4 | 11 | 32 | 16 |
| ***Staph. epidermidis*** | 4 | 1 | 5 | 3 | 3 | 16 | 8 |
| ***Proteus* spp.** | _ | 3 | 6 | _ | 3 | 12 | 6 |
| ***Salmonella* sp.** | _ | _ | 1 | _ | _ | 1 | 0.5 |
| ***Streptococcus* spp.** | _ | _ | _ | 5 | 1 | 6 | 3 |
| ***Citrobacter* sp.** | _ | _ | _ | _ | 1 | 1 | 0.5 |
| ***Candida spp.*** | _ | _ | _ | _ | 2 | 2 | 1 |
| **Total** | 25 | 50 | 50 | 25 | 50 | 200 | 100 |

Twenty antibiotics were used in this study. These antibiotics were selected as they are widely described by doctors and for in and out patient of hospitals. The pattern of total antibiotics susceptibility of pathogenic isolates as indicated in Figure 1 revealed that the most resistant bacterial isolates were isolates of *Pseudomonas* spp. (79%), followed by *Proteus* spp. (75.41%), followed by *Klebsiella* spp. (73.57%) and followed by *E.coli* (72.09%). It was found in general that more than 70% of all the four previous mentioned bacterial isolates were resistant to the tested antibiotics.

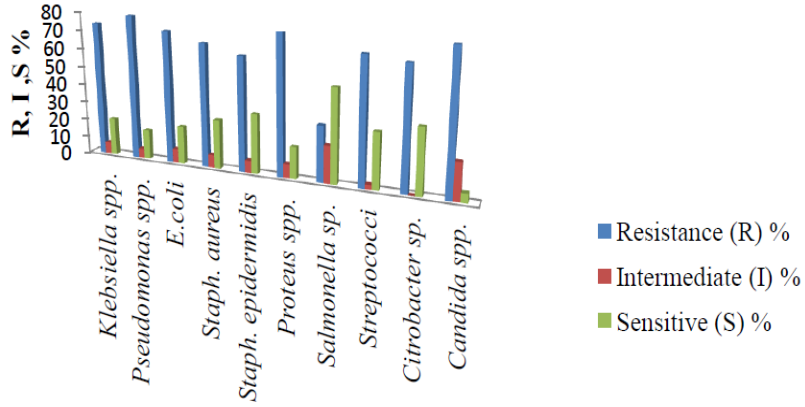


**Figure 1:** Pattern of total antibiotic susceptibility of clinical isolates.

The total pattern of resistance according to each antibiotic of the different antibiotics used was indicated in Table 2. In this table, The most efficient antibiotic was Amikacin (AK, aminoglycoside) by 48.5% sensitivity, 41.5% resistant strains and 10% intermediated isolates. The second most efficient antibiotic was gentamicin (CN, aminoglycoside) by 41.5% sensitive strains, 53% resistant strains and 5.5% intermediated strains, followed by the third most efficient antibiotic Norfloxacin (NOR, fluoroquinolone) by 41.5% sensitive isolates, 53.5% resistant strains and 5.0% intermediates. However, the worth antibiotic was Spectinomycin (SPT, aminocyclitol), in this case only 3.5% of the tested isolates were sensitive to this antibiotic as indicated in Table 2, followed by penicillins (Penicillin G (P), Amoxicillin/clavonic acid (AMC) and Amoxicillin (AX)). The four antibiotics showed more than or equal 90% resistance among the total clinical bacterial isolates tested in the present study.

**Table 2: Total Pattern of different antibiotic susceptibility of clinical bacterial isolates.**

| Antimicrobial agents | Resistance (R) | | Intermediate (I) | | Sensitive (S) | |
|---|---|---|---|---|---|---|
| | Total | % | Total | % | Total | % |
| **Streptomycin(S)** | 111 | 55.5 | 9 | 4.5 | 80 | 40 |
| **Cefepime(FEP)** | 174 | 87 | 17 | 8.5 | 9 | 4.5 |
| **Trimethoprim(TMP)** | 151 | 75.5 | 9 | 4.5 | 40 | 20 |
| **Tobramycin(TOB)** | 122 | 61 | 23 | 11.5 | 55 | 27.5 |
| **Pefloxacin(PEF)** | 123 | 61.5 | 27 | 13.5 | 50 | 25 |
| **Cefaclor(CEC)** | 170 | 85 | 9 | 4.5 | 21 | 10.5 |
| **Amikacin(AK)** | 83 | 41.5 | 20 | 10 | 97 | 48.5 |
| **Sparfloxacin(SPX)** | 102 | 51 | 14 | 7 | 84 | 42 |
| **Spectinomycin(SPT)** | 186 | 93 | 7 | 3.5 | 7 | 3.5 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Norfloxacin(NOR)** | 107 | 53.5 | 10 | 5 | 83 | 41.5 |
| **Cephradine(CE)** | 172 | 86 | 16 | 8 | 12 | 6 |
| **Penicillin G(P)** | 180 | 90 | _ | _ | 20 | 10 |
| **Aztreonam(ATM)** | 150 | 75 | 17 | 8.5 | 33 | 16.5 |
| **Cefotaxime(CTX)** | 172 | 86 | 24 | 12 | 4 | 2 |
| **Gentamicin(CN)** | 106 | 53 | 11 | 5.5 | 83 | 41.5 |
| **Piperacillin(PRL)** | 177 | 88.5 | _ | _ | 23 | 11.5 |
| **Amoxicillin/clavulanic acid(AMC)** | 180 | 90 | 7 | 3.5 | 13 | 6.5 |
| **Ciprofloxacin(CIP)** | 105 | 52.5 | 16 | 8 | 79 | 39.5 |
| **Ofloxacin(OFX)** | 104 | 52 | 12 | 6 | 84 | 42 |
| **Amoxicillin(AX)** | 180 | 90 | 15 | 7.5 | 5 | 2.5 |

$MIC_{50}$ (minimum inhibitory concentration)for each of aztreonam, meropenem, and amikacin were calculated for (12) clinical isolates of most MDR strains as shown in Table 4.The growth of 50% of the strains was inhibited by aztreonam(ATM), meropenem(MERO) and amikacin(AK) at different concentrations as indicated in Table 4. AK was the most effective antibiotic against *Klebsiella* sp. MAM-33, *Pseudomonas* sp. MAM-65, *Staph. epidermidis* MAM-71, *E. coli* MAM-104, *E.coli* MAM-105, *E. coli* MAM-125, *Streptococcus* sp. MAM-147, *Proteus sp.* MAM-171, *Klebsiella sp.* MAM-173 with $MIC_{50}$ 64, 64, 16, 4, 32, 64, 8, 4 and 16 µg/ml respectively. AK was the most efficient with the lowest $MIC_{50}$ (4 µg/ml) against *Proteus sp.* MAM-171 and *E. coli* MAM-104. In the case of MERO, $MIC_{50}$ for *Proteus sp.* Was 16 g/ml, which was >1024 for ATM antibiotic.

*E. coli* MAM-104 was the most sensitive bacterial strain among the 12 tested bacterial isolates tested. $MIC_{50}$ of E *coli* MAM-104 was 4, 16, 32 µg/ml of AK, MERO, ATM antibiotics respectively.

$MIC_{90}$ in Table 4 indicated the growth of 90% of the strains that were inhibited by ATM, MERO, and AK at different concentrations. Data shown for $MIC_{90}$ revealed that the most sensitive isolates were *Streptococcus* sp. MAM-147 followed by *Pseudomonas* sp. MAM-65. Both the two strains were sensitive for AK and MERO. Six out of 12 isolates achieved $MIC_{90}$ at concentrations ranging between 64 and 512 µg/ml of MERO.

**Table 4: Minimal inhibitory concentration (MIC50/MIC90) of different antibiotics against MDR clinical bacterial isolates.**

| Clinical strains code | MIC50/MIC90 (µg/ml) | | |
|---|---|---|---|
| | ATM | MERO | AK |
| ***Klebsiella*sp. *MAM-33*** | >1024/>1024 | >1024/>1024 | 64/>1024 |
| ***Klebsiella sp. MAM-61*** | 32/>1024 | >1024/>1024 | >1024/>1024 |
| ***Pseudomonassp. MAM-65*** | 512/>1024 | 128/512 | 64/512 |
| ***Staph. epidermidis MAM-71*** | >1024/>1024 | 32/>1024 | 16/>1024 |
| ***E. coli MAM-104*** | 32/>1024 | 16/64 | 4/>1024 |
| ***E. coli MAM-105*** | 1024/>1024 | 64/256 | 32/>1024 |
| ***E. coli MAM-125*** | 512/>1024 | 128/512 | 64/>1024 |
| ***Streptococcussp. MAM-147*** | 256/>1024 | 128/256 | 8/8 |
| ***Klebsiella sp. MAM-151*** | 1024/>1024 | 512/>1024 | >1024/>1024 |
| ***Proteus sp. MAM-171*** | >1024/>1024 | 16/256 | 4/>1024 |
| ***Klebsiella sp. MAM-173*** | >1024/>1024 | 32/>1024 | 16/>1024 |
| ***Staph. aureus MAM-193*** | 32/>1024 | 128/>1024 | 1024/>1024 |

After determination of $MIC_{50}$ and $MIC_{90}$ for each of the three antibiotics (AK, ATM, and MERO), double, triple, and four combinations were conducted between the previous antibiotics and in addition to the fourth antibiotic (tigecycline, TGC). The results of combinations (8 combinations), three of them were double, four of combinations were triple and one combination involved the four antibiotics (ATM, MERO, AK, and TGC) as indicated in Table 5. These results of 8 different antimicrobial combinations for 6 clinical isolates were better than the results of antibiotics alone without combinations because the growth of more than 95% of the strains was inhibited by this antimicrobial combinations.

The antibiotic combinations inhibiting the growth of clinical bacterial isolates by more than 95%in all combinations against all tested strains except in ATM/MRRO against *Streptococcus* sp. MAM-147 (88.2%), ATM/AK/TGC against *E. coli* MAM-105 (65.4%) and ATM/AK against *E. coli* MAM-105 (0.71%) which considered as the lowest inhibition growth percentage. An important observation had been recorded for *E. coli* MAM-105 that the double combination (ATM/ AK) was not efficient at all (0.71%). This means that Aztreonam and Amikacin were in antagonistic relation. However, the addition of the third

antibiotic (TGC) elevated the efficiency against *E. coli* MAM-105 from 0.71% to 65.4%. This means that addition of TGC enhanced the efficiency for the same clinical strain (MAM-105).

**Table 5: Percentage of growth inhibition of MDR pathogenic bacterial strains by antibiotic combinations.**

| Clinical isolatescode | Antimicrobial combinations | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| | | ATM/ MERO | ATM/ AK | MERO/ AK | ATM /MERO/ AK | ATM/ MERO/ TGC | ATM/ AK/ TGC | MERO/AK / TGC | ATM/ MERO/ AK/ TGC |
| | | GrowthO.D600nm /Growth inhibition% | | | | | | | |
| ***Klebsiella* sp. MAM- 33** | 0.828 | 0.017/ 98% | 0.045/ 95% | 0.015/ 98.1% | 0.013/ 98.4% | 0.014/ 98.3% | 0.021/ 97.5% | 0.025/ 97% | 0.030/ 96.4% |
| ***Klebsiella*sp. MAM- 61** | 1.127 | 0.031/ 97.2% | 0.028/ 97.5% | 0.032/ 97.1% | 0.030/ 97.3% | 0.031/ 97.2% | 0.035/ 97% | 0.020/ 98.2% | 0.023/ 98% |
| ***Pseudomon as* sp. MAM-65** | 1.137 | 0.019/ 98.3% | 0.015/ 98.7% | 0.033/ 97.1% | 0.021/ 98.2% | 0.006/ 99.5% | 0.007/ 99.4% | 0.031/ 97.3% | 0.033/ 97.1% |
| ***Staph.epidermidis* MAM-71** | 1.102 | 0.022/ 98% | 0.021/ 98.1% | 0.013/ 98.8% | 0.020/ 98.2% | 0.017/ 98.4% | 0.016/ 98.5% | 0.011/ 99% | 0.015/ 98.6% |
| ***E. coli* MAM-105** | 1.128 | 0.016/ 98.6% | 1.120/ 0.71% | 0.022/ 98% | 0.031/ 97.3% | 0.019/ 98.3% | 0.390/ 65.4% | 0.021/ 98.2% | 0.011/ 99% |
| ***Streptococcus*sp. MAM-147** | 1.283 | 0.152/ 88.2% | 0.009/ 99.3% | 0.031/ 97.6% | 0.004/ 99.7% | 0.012/ 99.1% | 0.007/ 99.5% | 0.013/ 99% | 0.009/ 99.3% |

## DISCUSSION

The clinical isolates wer collected from El-Gharbia Hospitals. These clinical isolates were 200 isolates, 144(72%) of them Gram-negative bacilli (GNB); 54(27%) Gram-positive cocci and two(1%) were *Candida* spp. GNB were *Klebsiella* spp. (42%), *E. coli* (15.5%), *Pseudomonas* spp. (7.5%), *Proteus* spp. (6%), *salmonella* sp. (0.5%) and *Citrobacter* sp. (0.5%).These results are much higher than that observed in Abidjan, as *Klebsiella pneumoniae* (14.9%), *Klebsiella oxytoca (8.1%), Pseudomonas aeruginosa* (5.3%), *Proteus mirabilis* (2.6%), *salmonella* spp. (0.4%) and *Salmonella Typhi* (0.1%). But *E. coli* (28.7%), is higher result than in that in the present study.[3] Severe multi drug-resistant (MDR) Gram-negative infections are increasing worldwide.[28] The Gram positive isolates were *Staph. aureus* (16%), *Staph. epidermidis* (8%) and *streptococcus* spp. (3%). Complicated skin and skin-structure infections are caused by Gram-positive cocci in the majority of cases.[29]

Twenty antibiotics were used in this study. (73.57%) of *Klebsiella* spp. were resistant to antibiotics that used, (6.19%) intermediate and (20.23%) sensitive. In the case of

*Pseudomonas* spp. (79 %) were the highest resistance, (5%) intermediate and (16%) sensitive. *Pseudomonas aeruginosa* is a uniquely problematic nosocomial pathogen because of its natural resistance to many drug families and its ability to acquire and rapidly develop resistance to multiple classes of antibiotics during the course of treating a patient.[30,31] However, (72.09%) of *E.coli* were resistant, (7.41%) intermediate and (20.48%) sensitive. In the case of *Staph. aureus*, (66.71%) resistant, (6.87%) intermediate and (26.40) sensitive. The overall burden of *staphylococcal* disease caused by antibiotic-resistant *S. aureus*, above all by the methicillin-resistant strains, is increasing in many countries, including Italy, in both healthcare and community settings.[32,33] *Proteus* spp. (75.41%) were resistant, (7.50%) intermediate and (17.08%) sensitive. *Staph. epidermidis* represented in resistance (61.56%), (6.56%) intermediate and (31.87) sensitive. *While Streptococcus* spp. (67.50%) were resistance, (2.50%) intermediate and (30%) sensitive. Rates of penicillin-non-susceptible (resistant + intermediately resistant) *Streptococcus pneumoniae* increased from <15% in 1997[34] to 61% in 2005.[35] *Citrobacter* sp. represented by (65%) resistance and (35%) sensitive. (75%) *Candida* spp. were resistant, (20%) intermediate and (5%) were the lowest sensitivity. On the other hand, *Salmonella* sp. represented the lowest resistance (30%), (20%) intermediate and also represented the highest sensitivity (50%).

The total pattern of resistance according to each antibiotic as indicated in Table 2 revealed that amikacin (AK) was the most effective antibiotic tested against most of the bacterial species, 48.5% of the tested clinical isolates were sensitive to AK. This antibiotic is a class of aminoglycosides which is considered as one of non-β- lactam antibiotics. These results were in agreement with that recorded by Abo-State et al.[5] They recorded that imipenem (64.28%) was the most effective antibiotic tested against the bacterial species isolated from hospitals in Cairo, Egypt, followed by amikacin (45.23%). Abo-State et al.[6] found that the clinical isolates have been investigated against 20 antibiotics. Eleven of these isolates were resistant to 20 of tested antibiotics out of 12 isolates, while the other isolate was resistant to 19 antibiotics. On the other hand, 93% was the highest percentage resistance of the clinical isolates against spectinomycin (SPT) that was the worth antibiotic as only 3.5% of the tested isolates were sensitive to this antibiotic. SPT is a class of aminocyclitols that is also one of non-β-lactam antibiotics. This followed by 90% were resistant to each of pencillin G (P), amoxicillin/clavonic acid (AMC) and amoxicillin (AX). Soon after the clinical introduction of penicillin G in early 1940, the problem of antibiotic-resistant bacteria emerged.[36] P (belong to subclass Classic penicillin) and AX (belong to subclass aminopenicillin) are the

class of pencillin according to Mascaretti.[37] But AMC is one of a class β- Lactam/β-lactamase inhibitor combinations. And each of P, AX and AMC is β-lactam antibiotic. Patients with cystic fibrosis (CF) have a higher prevalence of allergic reactions to one or multiple antibiotics, especially betalactams, thought to be due in part to multiple and repeated exposures. The worldwide prevalence of beta- lactam allergy in CF patients has been reported as high as 36%.[38] More than 50% were resistant to streptomycin (S), sparfloxacin (SPX), norfloxacin (NOR), gentamicin (CN), ciprofloxacin (CIP) and ofloxacin(OFX) that are non-β-lactam antibiotics. S and CN are part of class aminoglycosides. Aminoglycosides are a group of antibacterial antibiotics have a bactericidal activity against aerobic Gram- negative rods, including *Pseudomonas* spp.[39] While SPX, NOR, CIP and OFX are belonged to subclass fluoroquinolone and class quinolones. These classes were less effective against clinical isolates tested. When this present study is compared to another study in Abidjan, Moroh et al.[3] found that very high rates of resistance to amoxicillin, tetracycline and trimethoprim/ sulfamethoxazole were observed (close to 80%). Other antibiotics maintained their relative activity, such as ceftazidime, tobramycin, cefotaxime, aztreonan, polymyxin and especially netilmicin (3%). These results were observed both for Gram-positive isolates (*Staph. aureus)* and for GNB (*Klebsiella* spp., *E. coli*, *Pseudomonas* spp., *Proteus* spp., *Salmonella* spp. *Enterobacter* spp. and *Acinetobacter* spp.) whatever they came from in- or outpatients. As indicated in Table 3, it was found from the beginning of the collection period to the end of collection period that MDR bacterial isolates 186 isolates out of 200 (93%), these MDR involved 59 XDR isolates which represented 29.5%. The sensitive isolates were found to be 14 isolates (7%) from all the isolated strains. Controlling the spread of multi- or extensively drug-resistant bacteria (MDR or XDR) includes a dual strategy for reducing antibiotic prescriptions and preventing their spread from patient carriers.[40] This means that these clinical bacterial isolates are not only MDR bacteria but also eXDR (extensively drug resistant) bacteria.

**Table 3: Multi- or extensively drug-resistance of the pathogenic isolates.**

| *Pathogenic isolates* | Multi-Drug Resistance (MDR) | extensively drug resistance (XDR) | Sensitive (S) Bacteria | Total |
|---|---|---|---|---|
| ***Klebsiella spp.*** | 57 | 24 | 3 | 84 |
| ***Pseudomonasspp.*** | 8 | 7 | 0 | 15 |
| ***E.coli*** | 18 | 10 | 3 | 31 |
| ***Staph. aureus*** | 22 | 8 | 2 | 32 |
| ***Staph. epidermidis*** | 9 | 4 | 3 | 16 |
| ***Proteus spp.*** | 7 | 4 | 1 | 12 |
| ***Salmonella sp.*** | 0 | 0 | 1 | 1 |
| ***Streptococcus* spp.** | 3 | 2 | 1 | 6 |
| ***Citrobacter* sp.** | 1 | 0 | 0 | 1 |
| ***Candida* spp.** | 2 | 0 | 0 | 2 |
| **Total** | **127** | **59** | **14** | **200** |

$MIC_{50}$ (minimum inhibitory concentration) values to aztreonam(ATM), meropenem(MERO) and amikacin(AK) for12 clinical isolates of most MDR strains as shown in Table 4 indicated MERO and AK were best than ATM because most MDR strains need to concentration higher than 1024µg/ml from ATM to can inhibit the growth of 50% of the strains. Nine (9) out of twelve (12) isolates were sensitive to AK and $MIC_{50}$ of AK was the lowest concentration (the most efficient antibiotic) when compared with the two other antibiotics (ATM and MERO). This results confirmed the previous results of the present study that AK was the most efficient antibiotics. Amikacin has the largest spectrum among aminoglycosides.[41] But $MIC_{90}$ (minimum inhibitory concentration) values to the same three antibiotics ATM, MERO and AK for the same 12 clinical isolates of most MDR strains as shown in Table 4 indicated that the most sensitive isolates were *Streptococcus* sp. MAM-147 followed by *Pseudomonas* sp. MAM-65. Both the two strains were sensitive for AK and MERO. Six out of 12 isolates achieved $MIC_{90}$ at concentrations ranging between 64 and 512 µg/ml of MERO. Meropenem is important for management of postneurosurgical meningitis.[42] According to $MIC_{50}$ determination study, combination therapy may be the solution until new antibiotics become available. No standard MIC has been established for tigecycline (TGC) against MDR strains. However, values of 32 µg/ml for this drug are reported to indicate rapid bactericidal activity against MDR strains. Combinations of tigecycline (TGC) with other antibiotics are reported to be synergistic against MDR strains. Tigecycline, the first member of the glycylcyclines group of antibiotics with good in vitro activity against carbapenem resistant *Klebsiella pneumonia*.[43,44]

After determination of $MIC_{50}$ and $MIC_{90}$ for each of the three antibiotics (AK, ATM and MERO), double, triple, and four combinations were conducted between the previous antibiotics and in addition to the fourth antibiotic (Tigecycline, TGC). Combination therapy provides a useful therapeutic approach to overcome resistance until new antibiotics become available.[45]

The results of 8 different antimicrobial combinations of four antimicrobial drugs for 6 clinical isolates are better than the results of antibiotics alone without combinations because the growth of more than 95% of the strains were inhibited by these antimicrobial combinations except at the double combination (ATM/ AK) and the triple combination (ATM/ AK/ TGC) against the pathogenic isolate *E. coli* MAM-105 and the double combination (ATM/ MERO) for *Streptococcus* sp. MAM-147. According to Rahal, combined antimicrobial therapies can be used in clinical infections caused by bacterial strains that are susceptible to one or more antibiotics, or are resistant to all available antimicrobials. One desirable effect of the combination of antimicrobial agents may be to prevent the development of resistance to the active antibiotic by means of the addition of an inactive agent.[46] The result of aztreonam(ATM)/ amikacin(AK) combination for *Pseudomonas* sp. MAM-65 was close to what was found by Kataoka, as The result of aztreonam(ATM)/ amikacin(AK) combination for *Pseudomonas aeruginosa* inhibited growth by percentage reach to 81.3%.[47] The ATM/MERO/AK/TGC combination achieved growth inhibition by more than 95%. That close to the combination (AK/IPM/TIG/FEP) that was the only combination which achieved more than 90% killing against all of the isolates.[6]

## CONCLUSION

Gram negative bacilli (GNB) were the predominant among clinical bacterial pathogens. amikacin(AK) was the most effective antibiotic against clinical pathogens isolated from patients of El-Gharbia Governorate. In general, aminoglycoside antibiotic agent was the most effective antibiotic family in the present study. $MIC_{50}$ results confirmed the result of susceptibility test, that AK was the most effective antibiotic agent at lower concentrations. The combination therapy is better than the mono therapy for MDR and eXDR clinical bacterial isolates.

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