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Efficacy of Amikacin and Imipenem Against Multi-Drug Resistant Gram-Negative Bacteria Isolated from Wound Infections, Egypt

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ABSTRACT

Background and Aim: Gram-negative pathogens are considered the common cause of wound infections associated with increased mortality and morbidity rates. Antibiotics combination has been used to overcome this problem. In this study, we identify Gram-negative pathogens found in wound infections and assess the in-vitro efficacy of a combination of amikacin and imipenem against the resistant isolated pathogens.

Materials and Methods: One hundered fifty gram-negative bacteria were collected from two hundered patients suffering from different wound infections. Patients attended Minia University Hospitals,Egypt at period from January 2019- January 2020 and they were followed up periodically as a routine work in the hospitals. Swabs streaked on various media as Nutrient agar, MacConkey agar, Eosin methylene blue agar and cetrimide agar. The antimicrobial susceptibility of the identified pathogens was tested using the Kirby-Bauer method. Conventional PCR was used to detect the prevalence of *bla-$_{IMP}$* and AAC (6')-Ib genes. The effect of the tested combination was assessed by checkerboard technique and time-killing assay.

Results: *Escherichia coli* 38.6% was the most common isolated pathogen, followed by *Proteus spp* 30%, *P. aeruginosa* 21.4%, *Klebsiella spp.* 5.7%, and *Acinetobacter baumannii* 4.3%. The isolates were completely resistant to Ampicillin/sulbactam, Amoxicillin/clavulanic, Cephalothin, Cefadroxil, Ciprofloxacin, Ceftazidime and Ofloxacin. *Bla-$_{IMP}$* was detected in all *Klebsiella spp.*, *E. coli* (85.2%), *A. baumannii* (66.7%), *Proteus spp.* (38.1%) and *P. aeruginosa* (33.35%). *aac(6')-Ib* was detected among *E. coli, P. aeruginosa* and P*roteus spp. The* Checkerboard test showed a significant decrease in bacterial count in the presence of combination indicating a synergistic effect with FICIs ≤0.5. Time-kill assay showed a significant decrease in the bacterial count after 12h.

Conclusion: The studied combinations of antibiotics showed synergistic effects against the tested Gram-negative bacteria which can help in the control and treatment of serious wound infections.

Keywords: Gram-negative bacteria, imipenem, amikacin, Time kill curve, checkerboard assay, drug combination, synergism

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1. Introduction

Skin is considered the most significant barrier against any pathogens **(1)**. If any pathogen transfers through this barrier, infections will occur **(1)**. Wounds may be defined as any injury, damage, or break in the skin surface **(1)**. It may arise accidentally from a surgical incision or induced due to trauma or due to

disease as in diabetic foot (2). The induced trauma includes hospital-acquired wound infection established surgically or due to the use of intravenous medical devices (3). Hospital-acquired wound infections are considered the leading cause of nosocomial infections, prolonging hospital stays and increasing healthcare costs (3).

Most wound infections are classified into two categories including skin and soft tissue infections (4). Erythrasma, cellulitis, folliculitis, erysipelas, and impetigo are the most common skin infections (5). Dermatomycoses are skin infections caused by fungi and yeast (5). *Candida albicans*, Microsporum, Epidermophyton, Trichophyton, and Malassezia species are the most common fungal organisms (6).

The Pathogens obtained from surgical wound infections vary depending on the surgery performed (7). *Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Enterococci Staphylococcus aureus/MRSA Streptococcus pyogenes,* and *Corynebacterium spp* are the most common organisms found in wound infections (8). *Staphylococcus aureus* is the most dominant source of infection present during clean surgical procedures (9). Bacterial colonization may hinder wound healing if the bacterial load is greater than 10^5 organisms/g of tissue. Also, the type of bacteria seemed to inhibit wound healing and immune response (10). Culture is performed after a diagnosis of wound infection in order to recognize the pathogenic organisms and to choose the proper antibiotic therapy (8). Systemic antibiotics are preferred more than topical antibiotics in case of infected or colonized wounds (11). Nowadays, the emergence of resistance increases the need to use antimicrobial combinations to overcome this problem (11).

This study aims to identify the most predominant Gram-negative pathogens in wound infections, their resistance profiles to the most used medications in the Egyptian market, and assess drug combination between β-lactams (imipenem)/aminoglycosides (amikacin) in the treatment of severe wound infections.

2. Materials and Methods

Bacterial Isolates

One hundered fifty isolates of gram negative bacteria were found in 200 clinical samples collected from different patients suffering from wound infections (wound exudates, abscess exudates, and burn exudates). Patients were attending Minia University Hospital, Egypt from (January 2019-January 2020). Samples were streaked on nutrient agar, blood agar, MacConkey agar, and cetrimide agar. All inoculated cultures were grown at 37°C for 24 hr. Growth was examined both microscopically and biochemically (12).

Antimicrobial Sensitivity Test

The antimicrobial sensitivity test was conducted using Kirby, Bauer (13) disc diffusion method using different antimicrobial agents commonly used in the Egyptian market based on CLSI (2018). Antimicrobial agents used were gentamycin (10µg), Cefepime (30 µg), ceftazidime (30µg), meropenem (10µg), aztreonam (30µg), imipenem (10µg), amikacin (30µg), ofloxacin (10µg), ciprofloxacin (5µg), tobramycin (10µg), piperacillin (100µg), norfloxacin (10µg), levofloxacin (5µg), and piperacillin/tazobactam (10µg). The identified resistant isolates were tested by agar dilution method to investigate minimum inhibitory concentrations (MICs) for amikacin and imipenem according to recommendations and interpretative criteria for the Clinical and Laboratory Standards Institute (14). For better comparison, the MICs for 50% of isolates (MIC_{50}) and 90% of isolates (MIC_{90}) were determined.

Distribution of *bla-IMP* and aac (6')-Ib among the Tested Isolates

DNA was isolated from a culture that had been left overnight by the method described by Wilson, 1989 (15). The amplification was conducted with 25µL PCR reaction mixture containing (12.5µL Master mix, (200-400ng) DNA sample, Nuclease free water to 25 µL, 1 µL (20 pmol), for each forward and reverse primers). PCR cycling conditions are indicated in Table 1. The amplified product was analyzed after electrophoresis on a 2% agarose gel in TBE solution, stained with ethidium bromide and visualized using a UV transilluminator. The product of *bla-IMP* (488bp) and the product of aac (6')-Ib (365bp) was assessed by using a 1000-bp DNA ladder.

Table 1. The primers sequences include (16, 17)

Gene	Primer sequence (5'-3')	Annealing temperature
blaIMP	F:CATGGTTTGGTGGTTCTTGT	59
	R:ATAATTTGGCGGACTTTGGC	
Aac(6')-Ib	F:AGTACTTGCCAAGCGTTTTAGCGC	51
	R:CATGTACACGGCTGGACCAT	

Checkerboard Synergy Test

The synergistic action of the tested antibiotics combinations was determined by the checkerboard synergy test depending on micro-dilution susceptibility testing of imipenem and amikacin each alone and in combination. Each drug was evaluated at dilutions ranging from 0.03 to 64 µg/mL. The inoculum which obtained from colonies grown on MHA overnight. The effect of the studied combinations on microbial growth was measured using fractional inhibitory concentration (FIC).

The formula for calculating FIC index (FICI):

FIC= FIC of drug A+FIC of drug B; **FIC of drug A**= MIC of drug A in combination with drug B / MIC of drug A only; **FIC of drug B**= MIC of drug B in combination with drug A / MIC of drug B only. Synergism determined as FIC index of <0.5; Antagonism represented when FIC index of >4 and, FIC index 0.5 < FICI < 4 known as indifference **(18)**.

Time-killing Assay

The *in-vitro* bactericidal assessment of amikacin and imipenem detected by Time–kill curves. With a starting inoculum of 1.5×10^8, the test was conducted using concentrations of 0.5xMIC, 1xMIC, 2xMIC, and 4xMIC for each antibiotic alone and in combination. Tubes incubated at 37°C. Aliquots were obtained at 0, 2, 4, 8, 12, and 24 h, serially diluted by plating 10-fold dilutions on Muller-Hinton agar (BD Diagnostics, Franklin Lakes, NJ). The number of colonies was counted after 24h incubation at 37°C **(19)**. Bacteriostatic activities represented as ≥ 2 $\log_{10}$, but < 3 $\log_{10}$ reductions, and bactericidal activities indicates ≥ 3 $\log_{10}$ reductions in CFU/mL at 24 hours relative to the starting inoculum, where synergy seemed as a 2 $\log_{10}$ reduction in CFU/mL when using the drug combination, relative to the most active drug. Each experiment was repeated three times **(20)**

3. Results

Seventy Gram-negative isolates were detected in wound infections. *E. coli* was the most prevalent species (27 isolates, 38.6%), followed by *Proteus spp* (30%), *P. aeruginosa* (21.4%), *Klebsiella spp.* (5.7%) and *A. baumannii* (4.3%) (Table, 2).

Table 2. Prevalence of gram-negative pathogens among wound infections

Source of infections	Total number of isolates	*E. coli*	*P. aeruginosa*	*Proteus spp.*	*Klebsiella spp.*	*Acinetobacter baumannii*
Wounds	70	27 (38.6%)	15 (21.4%)	21 (30%)	4 (5.7%)	3 (4.3%)

The antibiotic resistance pattern among the isolated microorganisms (Figure 1) showed that Ampicillin/sulbactam was completely inactive against *P. aeruginosa, Proteus spp., Klebsiella spp and A. baumannii.* Amoxicillin/clavulanic was completely resistant against *E. coli*, *A. baumannii and Klebsiella spp.* Cephalothin was viewed as 100% resistant against *P. aeruginosa and A. baumannii.* Cefadroxil was completely resistant to *Klebsiella spp and A. baumannii*. Also, Ciprofloxacin had 100% resistant to *A. baumannii.* Ceftazidime and Ofloxacin were completely resistant to *Klebsiella spp.*

Determination of MIC, MIC_{90} and MIC_{50} of Amikacin and Imipenem

E. coli was highly resistant to imipenem and amikacin (81.5% and 55.6%, respectively). While *A. baumannii* showed no resistance to amikacin. On the other hand, *P. aeruginosa, proteus spp*. and *Klebsiella spp.* showed low resistance for both imipenem and amikacin as shown in Tables 3 & 4. MIC_{90} and MIC_{50} were used for better comparison.

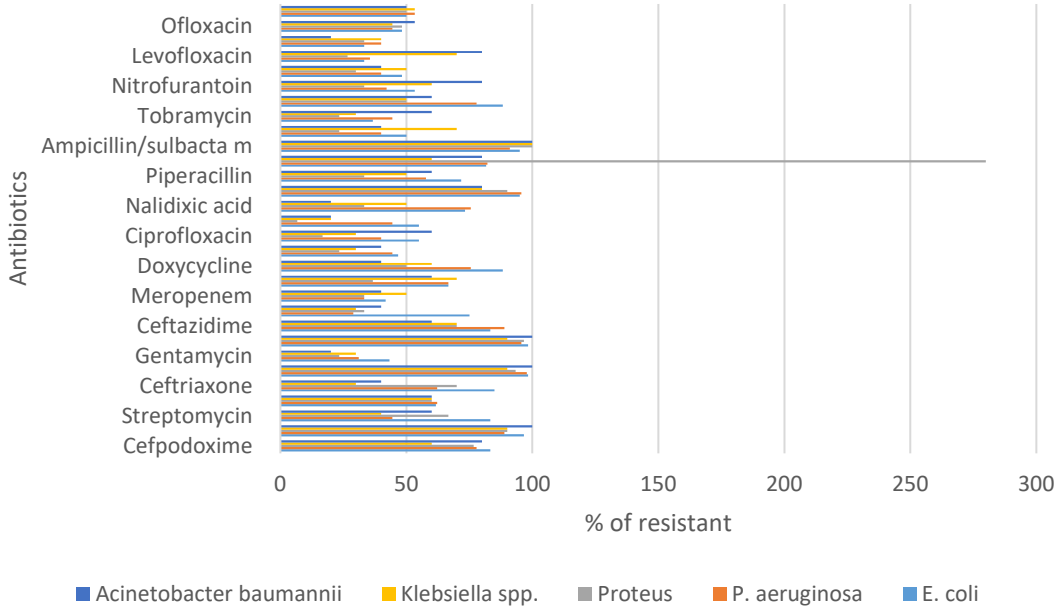


Figure 1. Antibiotics resistance pattern of the tested microorganisms

Table 3. MIC, MIC_{90} and MIC_{50} of amikacin against the tested isolates

Micro-organisms	Total no. of isolates	MIC_{90} (µg/ml)	MIC_{50} (µg/ml)	No. of Resistant isolates	%*
E. coli	27	512	32	22	81.5
P. aeruginosa	15	256	64	5	33.3
Proteus spp.	21	256	32	7	33.3
Klebsiella spp.	4	64	64	2	50
Acinetobacter baumannii	3	32	32	1	33.3

* Percent correlated to the number of resistant isolates\

Table 4. MIC, MIC_{90} and MIC_{50} of imipenem against the tested isolates

Micro-organisms	Total no. of isolates	MIC_{90} (µg/ml)	MIC_{50} (µg/ml)	No. of Resistant isolates	%*
E. coli	27	512	32	22	81.5
P. aeruginosa	15	256	64	5	33.3
Proteus spp.	21	256	32	7	33.3
Klebsiella spp.	4	64	64	2	50
Acinetobacter baumannii	3	32	32	1	33.3

* Percent was correlated to the number of resistant isolates

Distribution of bla_{IMP} and aac(6')-Ib Genotype Among Isolates

bla-IMP was found in *Klebsiella spp*. (100%), followed by *E. coli* (85.2%), *A. baumannii* (66.7%), *Proteus spp.* (38.1%) and *P. aeruginosa* (33.35%). aac(6')-Ib was highly distributed among *E. coli, P. aeruginosa* and *Proteus spp.* (70.4%, 46.7% and 4.8%, respectively) (Table 5 & Figures 2 and 3).

Table 5. Distribution of Bla_{IMP} and AAC *(6′)-Ib* genotype among the tested isolates

Name of organism	No. of the isolates in each infection	*bla_{IMP}* (%)*	*AAC (6′)-Ib (%)* *
E. coli	27	23 (85.2%)	19 (70.4%)
P. aeruginosa	15	5 (33.35%)	7 (46.7%)
Proteus spp.	21	8 (38.1%)	1 (4.8%)
Klebsiella spp.	4	4 (100%)	-
Acinetobacter baumannii	3	2 (66.7%)	-

***Percent was correlated to the no. of each isolate**

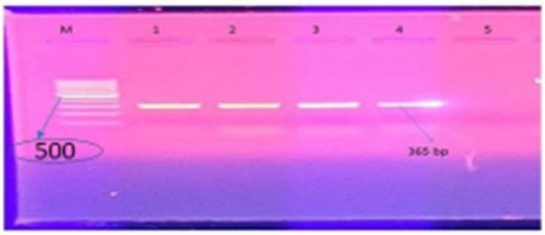


Figure 2. Agarose gel showing PCR-amplified products of *AAC (6′)-Ib* (365bp). Lane M: 100 bp DNA ladder; lane 1: *AAC (6′)-Ib*; lane 2: *AAC (6′)-Ib*; lane 3: *AAC (6′)-Ib*,; lane 4: *AAC (6′)-Ib* and lane 5:No band

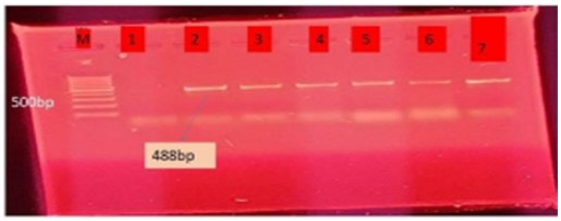


Figure 3. Agarose gel showing PCR-amplified products of *bla_{IMP}* (488bp). Lane M: 100 bp DNA ladder; lane 1:No band :*bla_{IMP}*; lane 2: *bla_{IMP}*; lane 3: *bla_{IMP}*; lane 4: *$bla_{IMP;}$* lane 5: *bla_{IMP}*; lane 6: *bla_{IMP}* and lane 7: *bla_{IMP}*

The combined activity between amikacin and imipenem versus the tested isolates by checkerboard technique

Amikacin and imipenem were assessed in combination because they had good activity against the tested isolates. Also, we expect that this combination may have synergistic activity against MDR isolates due to the difference in mechanisms of action between both antibiotics. Our results showed that the antibacterial combination against resistant strains lowered the MICs of each drug and the efficacy of the tested antibiotics increased. FIC index of both drugs varied from 0.01 to 0.5 indicating synergistic activity for the combination. The effect of amikacin and imipenem combination against resistant strains found to lower MICs for both amikacin and imipenem from 1024µg/ml to 32µg/ml (FIC_{index} 0.06) Table 6.

Table 6. The combined activity between imipenem and amikacin versus the tested isolates:

Name of bacteria	MIC (µg/ml)				FIC index	Outcome
	Amikacin alone	Imipenem alone	Combination amikacin + imipenem			
***E. coli* (No.1)**	1024	1024	32	32	0.06	Synergistic
***E. coli* (No.2)**	1024	512	32	32	0.09	Synergistic
***E. coli* (No.3)**	512	512	16	4	0.03	Synergistic
***E. coli* (No.4)**	512	128	32	8	0.125	Synergistic
***E. coli* (No.5)**	256	128	32	8	0.187	Synergistic
***E. coli* (No.6)**	256	64	32	4	0.187	Synergistic
***E. coli* (No.7)**	128	64	1	0.5	0.016	Synergistic
***E. coli* (No.8)**	128	64	8	4	0.125	Synergistic
***E. coli* (No.9)**	64	32	1	1	0.047	Synergistic
***E. coli* (No.10)**	64	32	1	0.5	0.03	Synergistic
***E. coli* (No.11)**	32	32	1	0.5	0.05	Synergistic

Name of bacteria			MIC (µg/ml)		FIC index	Outcome
***E. coli* (No.12)**	32	16	0.5	2	0.14	Synergistic
***E. coli* (No.13)**	16	8	0.5	2	0.3	Synergistic
***E. coli* (No.14)**	16	4	0.5	0.25	0.09	Synergistic
***E. coli* (No.15)**	16	4	1	0.5	0.19	Synergistic
***Ps* (No.1)**	1024	1024	32	32	0.06	Synergistic
***Ps* (No.2)**	512	512	32	8	0.078	Synergistic
***Ps* (No.3)**	256	256	4	1	0.019	Synergistic
***Ps* (No.4)**	128	128	32	8	0.3	Synergistic
***Ps* (No.5)**	64	64	0.5	1	0.023	Synergistic
***Proteus* (No.1)**	64	512	0.5	1	0.001	Synergistic
***Proteus* (No.2)**	64	16	0.5	1	0.07	Synergistic
***Proteus* (No.3)**	64	8	0.25	0.5	0.066	Synergistic
***Kl.* (No.1)**	64	128	2	1	0.04	Synergistic
***Kl.* (No.2)**	64	64	0.5	1	0.023	Synergistic

Time–kill studies

***Regarding E. coli* (No. 1) resistant to both imipenem and amikacin:** The initial bacterial count was 8.2 $\log_{10}$ CFU/ml. At 0.5xMIC there was no significant decrease in bacterial count except after 24hrs by the combination group to 7.46 $\log_{10}$ CFU/ml.

At 1xMIC the bacterial count decreased significantly for each drug alone and showed a significant decrease in colony count (bactericidal) by the tested combination to 3.5 log10 CFU/mL after 24h, indicating synergistic activity. At 2xMIC, a bacteriostatic effect was shown by 2.26 $\log_{10}$ CFU/mL reductions at 12h, indicating bacteriostatic activity. At 4xMIC no colony count found at 12h and 8h for each drug alone and in combination, respectively (Figure, 4).

For *Pseudomonas aeruginosa* (No. 1) resistant to both imipenem and amikacin.

The initial *in-vitro* bacterial count was 8.2 $\log_{10}$ CFU/mL, at 0.5xMIC bacterial count decreased to 7.48 $\log_{10}$ CFU/mL after 24h in case of imipenem and amikacin combination. At 1xMIC combination showed 3.2 $\log_{10}$ CFU/mL reductions which indicated bactericidal and synergistic compared with both drugs after 24h. At 2xMIC combination regimen showed bacteriostatic after 12h with 2.49 $\log_{10}$ CFU/mL reductions, bactericidal after 24h with 3.6 $\log_{10}$ CFU/mL reductions, where ≥ 2 $\log_{10}$ CFU/mL reductions after 24h had to be synergistic. At 4xMIC no colony count found after 24h and 12h in the presence of each drug alone and in combination (Figure, 5).

Regarding *Proteus* (No.1) resistant to both drugs

At 0.5xMIC, combination showed decrease in colony count to 8.03 $\log_{10}$ CFU/mL after 24h, 1xMIC decreased in case of combination to 5 $\log_{10}$ CFU/mL with 3 $\log_{10}$ CFU/mL reductions shown to be bactericidal and synergistic between both drugs. At 2xMIC, bacteriostatic activity was shown after 8h with 2.4 $\log_{10}$ CFU/mL reductions and bactericidal activity after 12h with 3.57 $\log_{10}$ CFU/mL reductions. At 4xMIC, combination showed bacteriostatic activity at 2h with 2.5 $\log_{10}$ CFU/mL reductions and showed bactericidal activity after 4h with 3.7 $\log_{10}$ CFU/mL reductions (Figure, 6).

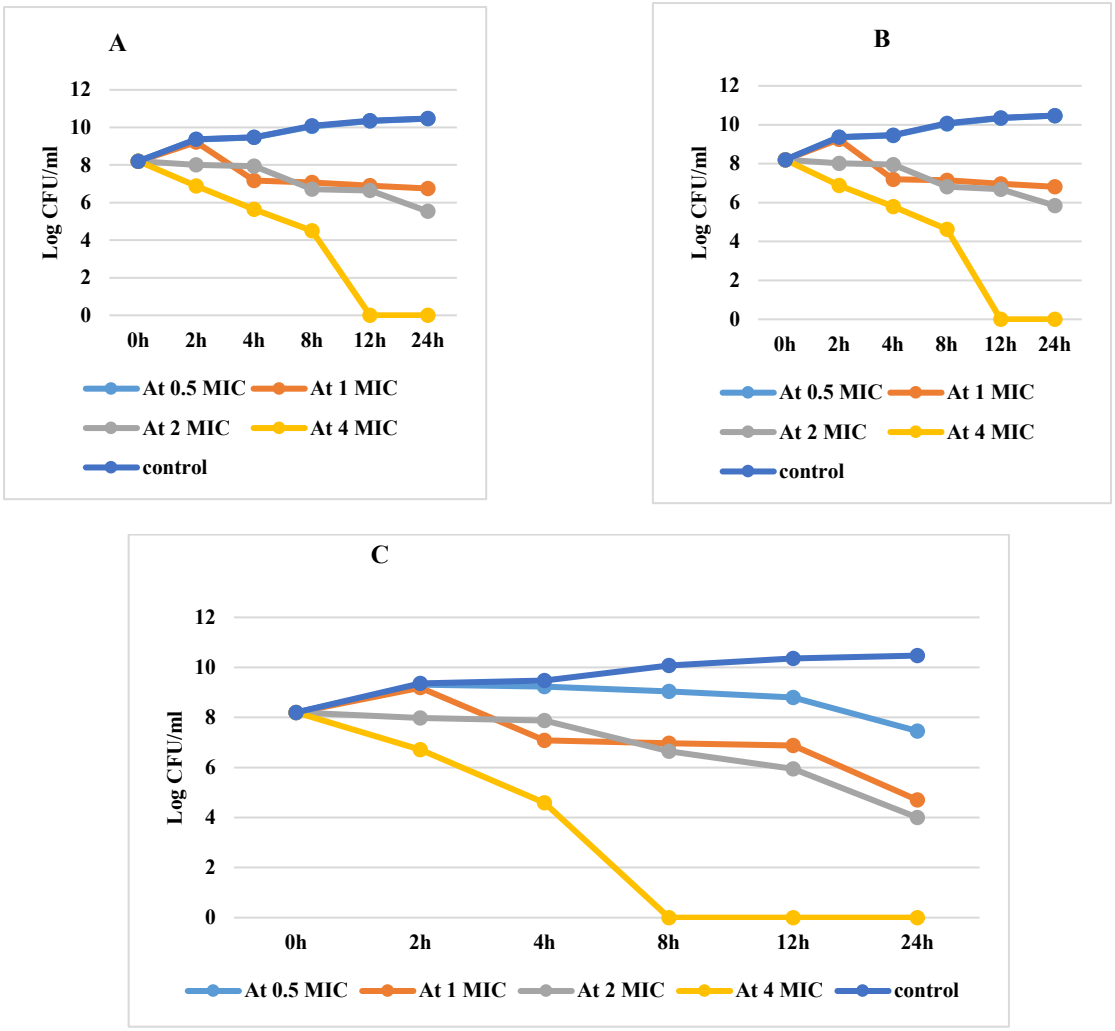
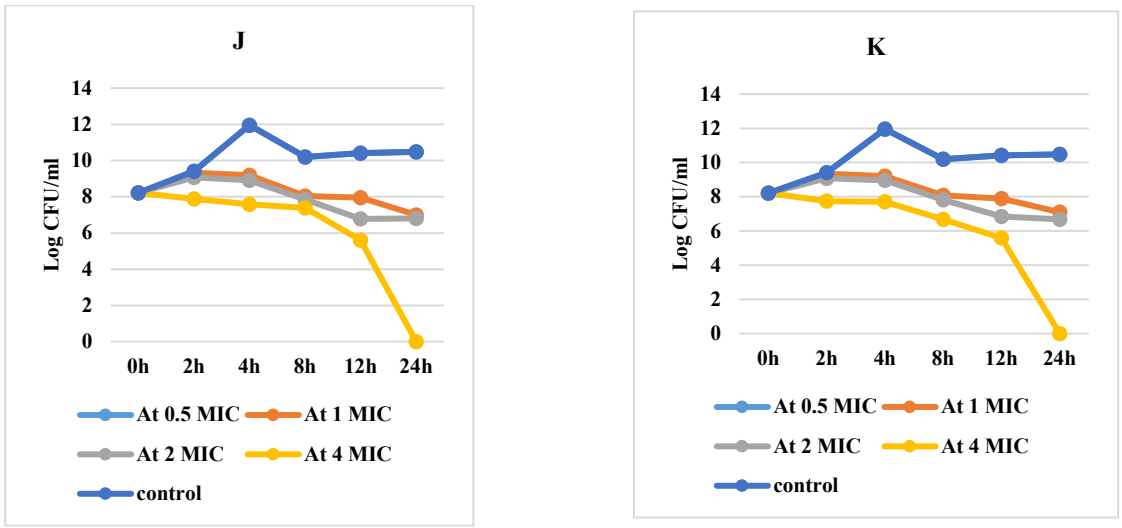


Figure 4. Time killing curve for *E. coli* **A**: Treated with amikacin in different concentrations, **B***:* Treated with imipenem in different concentrations, **C**: Treated with a combination in different concentrations



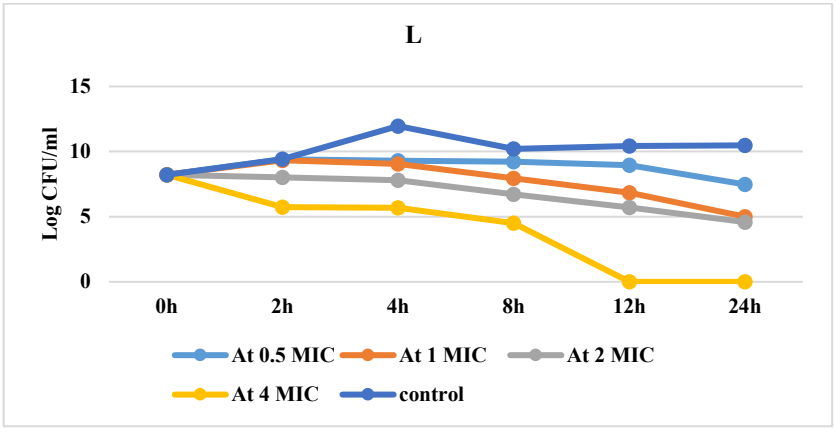


Figure 5. Time killing curve for *P. aeruginosa* **J:** Treated with amikacin in different concentration, **K:** Treated with imipenem in different concentration, **L**: Treated with combination in different concentration

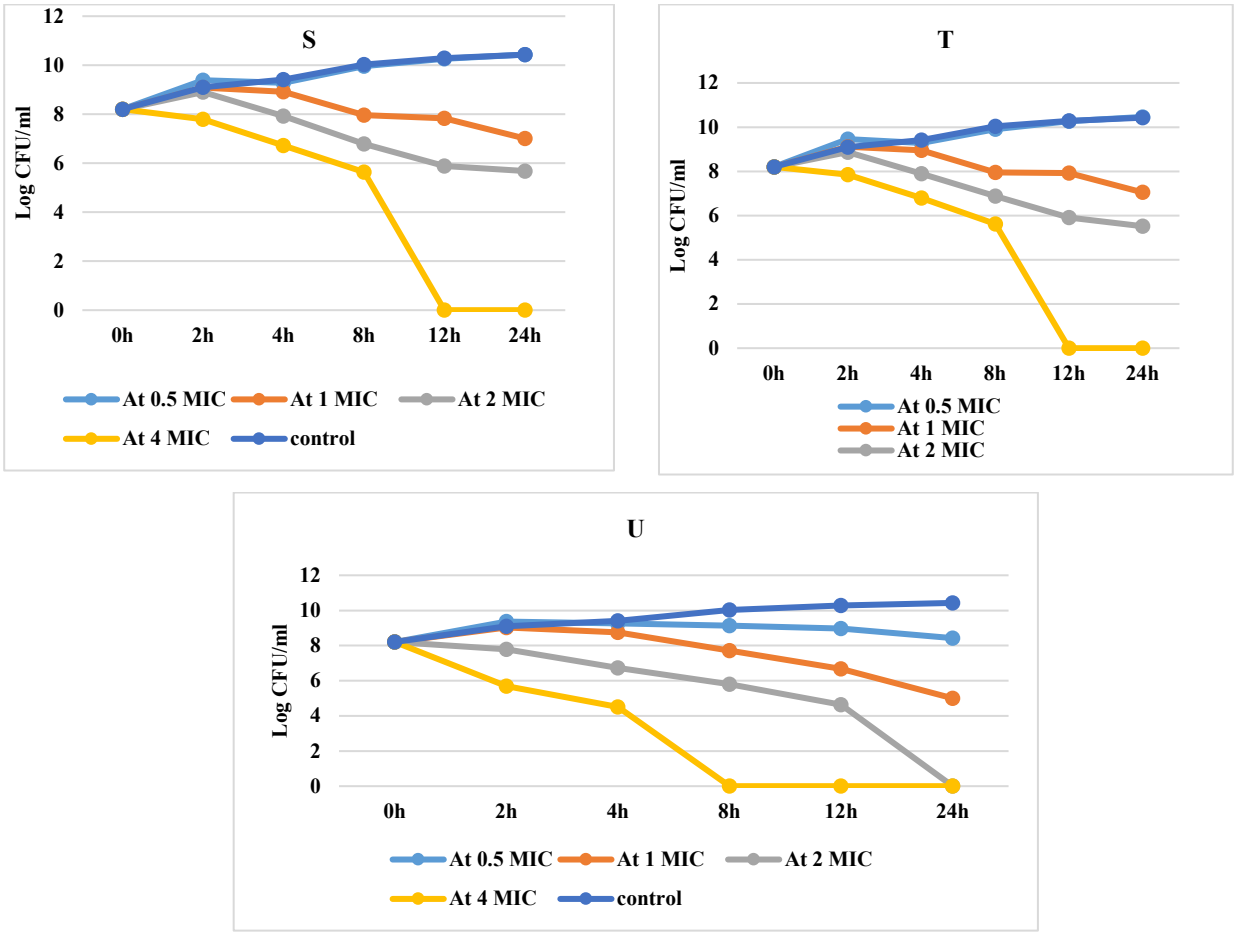


Figure 6. Time killing curve for *Proteus spp*. **S:** Treated with amikacin in different concentrations, **T:** Treated with imipenem in different concentrations, **U:** Treated with a combination of different concentration

The Case of *Klebsiella* (No.1) resistant to both drugs

At 0.5xMIC, the combination showed a decrease in the bacterial count to 8 $\log_{10}$ CFU/mL. At 1xMIC, the combination showed 3.35 $\log_{10}$ CFU/mL reductions indicating bactericidal and synergistic after 24h. At 2xMIC, the Combination decreased bacterial count after 4h with 2.27 $\log_{10}$ CFU/mL reductions showing bacteriostatic activity while bactericidal was observed after 8h with 3.6 $\log_{10}$ CFU/mL reductions. At 4xMIC combination showed bacteriostatic effect after 2h with 2.49 $\log_{10}$ CFU/mL reductions and showed bactericidal activity after 4h with 3.68 $\log_{10}$ CFU/mL reductions (Figure, 7).

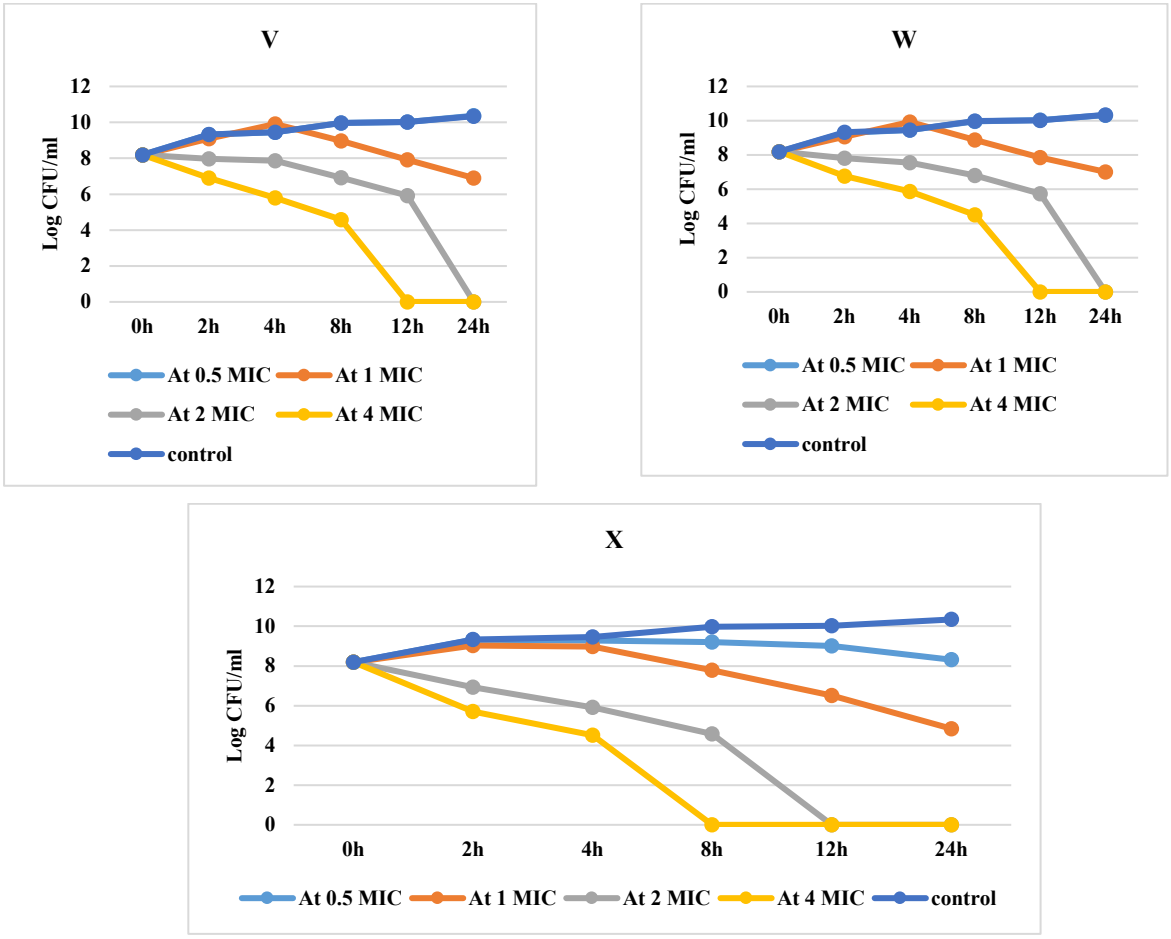


Figure 7. Time killing curve for *Klebsiella spp*. **V**: Treated with amikacin in different concentration, **W:** Treated with imipenem in different concentration, **X:** Treated with combination in different concentration

4. Discussion

Gram-negative bacterial wound infections play an important role in chronicity and slowing wound healing. These infections should be limited and managed by healthcare practitioners by suggesting suitable antibiotic treatments **(8)**.

In this study, 70 Gram-negative microbes were obtained from wounds showing signs of infections. The most predominant strains were *E. coli, Proteus spp., P. aeruginosa, Klebsiella spp.* and *A. baumannii* in agreement with Bhatt, Tandel **(21)** who stated that *E. coli, Proteus spp., P. aeruginosa, Klebsiella spp.* and *A. baumannii* were the most prevalent microbes isolated from wound swabs. Bessa, Fazii **(8)** and Gjødsbøl, Christensen **(22)** stated that *P. aeruginosa, Proteus mirabilis* and *E. coli* were the most common Gram-negative isolates isolated from wound infections.

Exposure to antimicrobial drugs for long periods is the most common cause widespread of resistance among Gram-negative bacteria **(23)**. In the present study, Gram-negative pathogen showed MDR to most of the antibiotics, to overcome this resistance, combination therapy has been used **(24)**. Previously, synergistic combinations of β-lactams and aminoglycosides were reported to overcome resistance caused by MDR Gram-negative bacteria by Lim, Lee **(25)**. The reason for synergism between both drugs is that both drugs act by a different inhibitory mechanism. Beta-lactam antibiotics such as imipenem, attach to penicillin-binding proteins (PBPS) and cause morphological changes in cells **(26)** whereas aminoglycosides such as amikacin, inhibit protein synthesis **(27)**. Other antibiotics' periplasmic target site penetration is aided by the permeabilizing impact. As a result, carbapenems in conjunction with an aminoglycoside are effective **(28)**. In our study Gram-negative isolates showed complete resistance to Ampicillin/sulbactam, Amoxicillin/ clavulanic, Cephalothin, Cefadroxil, Ciprofloxacin, Ceftazidime and Ofloxacin. A study done by Vena, Giacobbe **(29)**, it was found that Gram-negative pathogens were mostly resistant to cefepime, ceftazidime, ciprofloxacin, piperacillin/tazobactam, and colistin.

One of the most predominant genes of resistance among Gram-negative bacteria are *bla-$_{IMP}$* and AAC

(6')-Ib **(30)**. So, we must study the prevalence of these genes in isolated pathogens. Our findings showed that all *Klebsiella spp.* harbored *bla-IMP*, followed by *E. coli*, *A. baumannii*, *Proteus spp.* and *P. aeruginosa*. aac(6')-Ib were most common among *E. coli, P. aeruginosa* and *Proteus spp*. Elbadawi, Elhag **(31)** reported that 7 isolates out of 21 Gram negative bacteria harbored *bla-IMP* and Costello, Deshpande **(32)** stated that aac(6')-Ib was the predominant aminoglycoside modifying enzyme. The present study revealed that *in-vitro* activity of imipenem and amikacin combination showed synergistic action against most resistant Gram-negative pathogens. The combination of both drugs reported a significant decrease in bacterial count shown by the time-kill curve. Such a combination could be a promising therapy in treating lethal Gram-negative bacterial infections as it reduces the risk of monotherapy resistance and improves clinical treatment. The present study opposite to the study done by Mathe, Szabo **(18)** found that imipenem and amikacin alone had activities much better than their combination in the treatment of *Klebsiella.* In another study done by Rodríguez-Hernández, Pachón **(19)**, it was found that imipenem as monotherapy was much better than amikacin alone or IMP/AMK combination in the treatment of *A. baumannii*.

5. Conclusion

Amikacin and imipenem combination showed the best solution therapy against serious Gram-negative bacterial wound infections and this combination may combat life threatening or severe wound infections.

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Ethics Approval & Consent to Participate

The research was approved by the Research and Ethics Committee of the Review Board of Faculty of Pharmacy (Number:8/2021)

Authors' Contributions

Formal analysis: S.M.F. and A.O.E.-G.; investigation: A.F.A.; R.M.A.E-B .methodology, S.M.F.; project administration: A.O.E.-G. and A.F.A .; supervision: R.M.A.E.-B., A.O.E.-G. and S.A.; validation: S.M.F. and S.A.; visualization: A.F.A.; S.M.F Software: S.M.F.; writing original draft: S.M.F. and A.F.A.; writing review and editing: R.M.A.E-B., A.F.A.

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Conflict of Interest

The authors declared no conflict of interest.

Reference

1. Petruzziello A, Marigliano S, Loquercio G, Cozzolino A, Cacciapuoti C. Global epidemiology of hepatitis C virus infection: An up-date of the distribution and circulation of hepatitis C virus genotypes. World J Gastroenterol. 2016;22(34): 7824-40. [DOI:10.3748/wjg.v22.i34.7824] [PMID] [PMCID]
2. Cooke GS, Lemoine M, Thursz M, Gore C, Swan T, Kamarulzaman A, et al. Viral hepatitis and the Global Burden of Disease: a need to regroup. J Viral Hepat. 2013;20(9):600-1. [DOI:10.1111/jvh.12123] [PMID]
3. Mohamoud YA, Mumtaz GR, Riome S, Miller D, Abu-Raddad LJ. The epidemiology of hepatitis C virus in Egypt: a systematic review and data synthesis. BMC Infect Dis. 2013;13(1):288.[PMID] [DOI:10.1186/1471-2334-13-288] [PMCID]
4. Elgharably A, Gomaa AI, Crossey MME, Norsworthy PJ, Waked I, Taylor-Robinson SD. Hepatitis C in Egypt - past, present, and future. Int J Gen Med. 2017;10:1-6. [DOI:10.2147/IJGM.S119301] [PMID] [PMCID]
5. Ditah I, Ditah F, Devaki P, Ewelukwa O, Ditah C, Njei B, et al. The changing epidemiology of hepatitis C virus infection in the United States: National health and nutrition examination survey 2001 through 2010. J Hepatol. 2014;60(4):691-8. [DOI:10.1016/j.jhep.2013.11.014] [PMID]
6. Lu M, Li J, Rupp LB, Zhou Y, Holmberg SD, Moorman AC, et al. Changing trends in complications of chronic hepatitis C. Liver Int. 2018;38(2):239-47. [DOI:10.1111/liv.13501] [PMID] [PMCID]
7. de Oliveria Andrade LJ, D'Oliveira A, Melo RC, De Souza EC, Costa Silva CA, Paraná R. Association between hepatitis C and hepatocellular carcinoma. J Glob Infect Dis. 2009;1(1):33-7. [DOI:10.4103/0974-777X.52979] [PMID] [PMCID]
8. Bennett JE, Dolin R, Blaser MJ. Mandell, douglas, and bennett's principles and practice of

infectious diseases E-book: Elsevier health sciences; 2019.

9. Geddawy A, Ibrahim YF, Elbahie NM, Ibrahim MA. Direct acting anti-hepatitis C virus drugs: clinical pharmacology and future direction. J Transl int Med. 2017;5(1):8-17. [DOI:10.1515/jtim-2017-0007] [PMID] [PMCID]

10. Ji F, Wei B, Yeo YH, Ogawa E, Zou B, Stave CD, et al. Systematic review with meta-analysis: effectiveness and tolerability of interferon-free direct-acting antiviral regimens for chronic hepatitis C genotype 1 in routine clinical practice in Asia. Aliment Pharmacol Ther. 2018;47(5):550-62. [DOI:10.1111/apt.14507] [PMID]

11. Wei B, Ji F, Yeo YH, Ogawa E, Stave CD, Dang S, et al. Systematic review and meta-analysis: real-world effectiveness of direct-acting antiviral therapies in chronic hepatitis C genotype 3 in Asia. BMJ Open Gastroenterol. 2018;5(1): e000209. [DOI:10.1136/bmjgast-2018-000209] [PMID] [PMCID]

12. Liang TJ, Ghany MG. Current and future therapies for hepatitis C virus infection. N Engl J Med. 2013;368(20):1907-17. [DOI:10.1056/NEJMra1213651] [PMID] [PMCID]

13. Castillo I, Rodríguez-Iñigo E, López-Alcorocho JM, Pardo M, Bartolomé J, Carreño V. Hepatitis C Virus Replicates in the Liver of Patients Who Have a Sustained Response to Antiviral Treatment. Clin Infect Dis. 2006;43(10):1277-83. [DOI:10.1086/508198] [PMID]

14. Hetta HF, Mehta MJ, Shata MTM. Gut immune response in the presence of hepatitis C virus infection. World J Immunol. 2014;4(2):52-62. [DOI:10.5411/wji.v4.i2.52]

15. Mekky MA, Sayed HI, Abdelmalek MO, Saleh MA, Osman OA, Osman HA, et al. Prevalence and predictors of occult hepatitis C virus infection among Egyptian patients who achieved sustained virologic response to sofosbuvir/daclatasvir therapy: a multi-center study. Infect Drug Resist. 2019;12:273-9. [DOI:10.2147/IDR.S181638] [PMID] [PMCID]

16. Fallahi P, Ferri C, Ferrari SM, Corrado A, Sansonno D, Antonelli A. Cytokines and HCV-related disorders. Clin Dev Immunol. 2012;2012. [DOI:10.1155/2012/468107] [PMID] [PMCID]

17. Antonelli A, Ferrari SM, Ruffilli I, Fallahi P. Cytokines and HCV-related autoimmune disorders. Immunol Res. 2014;60(2):311-9. [DOI:10.1007/s12026-014-8569-1] [PMID]

18. Kim AY, Kuntzen T, Timm J, Nolan BE, Baca MA, Reyor LL, et al. Spontaneous Control of HCV Is Associated With Expression of HLA-B*57 and Preservation of Targeted Epitopes. Gastroenterology. 2011;140(2):686-96.e1. [DOI:10.1053/j.gastro.2010.09.042] [PMID] [PMCID]

19. Grakoui A, Shoukry NH, Woollard DJ, Han J-H, Hanson HL, Ghrayeb J, et al. HCV persistence and immune evasion in the absence of memory T cell help. Science. 2003;302(5645):659-62. [DOI:10.1126/science.1088774] [PMID]

20. Shoukry NH, Grakoui A, Houghton M, Chien DY, Ghrayeb J, Reimann KA, et al. Memory CD8+ T cells are required for protection from persistent hepatitis C virus infection. J Exp Med. 2003; 197(12):1645-55. [DOI:10.1084/jem.20030239] [PMID] [PMCID]

21. Gad YZ, Mouas N, Abdel-Aziz A, Abousmra N, Elhadidy M. Distinct immunoregulatory cytokine pattern in Egyptian patients with occult Hepatitis C infection and unexplained persistently elevated liver transaminases. Asian J Transfus Sci. 2012;6(1):24-8. [PMID] [PMCID] [DOI:10.4103/0973-6247.95046]

22. Flynn JK, Dore GJ, Hellard M, Yeung B, Rawlinson WD, White PA, et al. Maintenance of Th1 hepatitis C virus (HCV)-specific responses in individuals with acute HCV who achieve sustained virological clearance after treatment. J Gastroenterol Hepatol. 2013 (11):1770-81. [DOI:10.1111/jgh.12265] [PMID] [PMCID]

23. Kandeel A, Genedy M, El-Refai S, Funk AL, Fontanet A, Talaat M. The prevalence of hepatitis C virus infection in Egypt 2015: implications for future policy on prevention and treatment. Liver Int. 2017;37(1):45-53. [DOI:10.1111/liv.13186] [PMID] [PMCID]

24. Scheel TKH, Rice CM. Understanding the hepatitis C virus life cycle paves the way for highly effective therapies. Nat Med. 2013;19(7): 837-49. [DOI:10.1038/nm.3248] [PMID] [PMCID]

25. Abd Alla MDA, El Awady MK. Hepatitis C Virus RNA Strands Detection in Peripheral Blood Mononuclear Cells Legitimizes Virus Eradication in Negative Serum PCR Naïve and Post-treatment Patients. J Clin Transl Hepatol. 2017;5(1):1-8. [DOI:10.14218/JCTH.2016.00054] [PMID] [PMCID]

26. Hanno AFF, Mohiedeen KM, Alshayeb AF, Deghedy A. HCV RNA in peripheral blood mononuclear cells (PBMCs) as a predictor of the response to antiviral therapy in chronic hepatitis

C. Alexandria J Med. 2014;50(4):317-22. [DOI:10.1016/j.ajme.2013.05.004]

27. Pawełczyk A, Kubisa N, Jabłońska J, Bukowska-Ośko I, Caraballo Cortes K, Fic M, et al. Detection of hepatitis C virus (HCV) negative strand RNA and NS3 protein in peripheral blood mononuclear cells (PBMC): CD3+, CD14+ and CD19+. Virol J. 2013;10(1):346. [PMID] [PMCID] [DOI:10.1186/1743-422X-10-346]
28. De Marco L, Gillio-Tos A, Fiano V, Ronco G, Krogh V, Palli D, et al. Occult HCV infection: an unexpected finding in a population unselected for hepatic disease. PloS One. 2009;4(12):e8128. [DOI:10.1371/journal.pone.0008128] [PMID] [PMCID]
29. Pham TNQ, Coffin CS, Michalak TI. Occult hepatitis C virus infection: what does it mean? Liver Int. 2010;30(4):502-11. [DOI:10.1111/j.1478-3231.2009.02193.x] [PMID]
30. Aboalam H, Rashed H-A, Mekky M, Nafeh H, Osman O. Prevalence of occult hepatitis C virus in patients with HCV-antibody positivity and serum HCV RNA negativity. Curr Med Res Pract. 2016;1(2):12-6. [DOI:10.4103/2357-0121.192539]
31. Yousif MM, Fakhr AE, Morad EA, Kelani H, Hamed EF, Elsadek HM, et al. prevalence of occult hepatitis c virus infection in patients who achieved sustained virologic response to direct-acting antiviral agents. Infez Med. 2018;26(3): 237-43.
32. Abu Khadr NA, Nouh HH, Hanafi NF, Asser SL, Hussain YA. Secondary occult hepatitis C virus infection (HCV) in chronic HCV patients after treatment with sofosbuvir and daclatasvir. Int J Curr Microbiol App Sci. 2018;7(1):1357-65. [DOI:10.20546/ijcmas.2018.701.165]
33. Castillo I, Bartolomé J, Quiroga JA, Barril G, Carreño V. Diagnosis of occult hepatitis C without the need for a liver biopsy. J Med Virol. 2010;82(9):1554-9. [DOI:10.1002/jmv.21866] [PMID]
34. Cavalheiro Nde P, Filgueiras TC, Melo CE, Morimitsu SR, de Araújo ES, Tengan FM, et al. Detection of HCV by PCR in serum and PBMC of patients with hepatitis C after treatment. Braz J Infect Dis. 2007;11(5):471-4. [PMID] [DOI:10.1590/S1413-86702007000500006]
35. Radkowski M, Gallegos-Orozco JF, Jablonska J, Colby TV, Walewska-Zielecka B, Kubicka J, et al. Persistence of hepatitis C virus in patients successfully treated for chronic hepatitis C. Hepatology. 2005;41(1):106-14. [DOI:10.1002/hep.20518] [PMID]
36. Bernardin F, Tobler L, Walsh I, Williams JD, Busch M, Delwart E. Clearance of hepatitis C virus RNA from the peripheral blood mononuclear cells of blood donors who spontaneously or therapeutically control their plasma viremia. Hepatology. 2008;47(5):1446-52. [DOI:10.1002/hep.22184] [PMID]
37. Rahman MZ, Ahmed DS, Masud H, Parveen S, Rahman MA, Chowdhury MS, et al. Sustained virological response after treatment in patients with chronic hepatitis C infection--a five year follow up. Bangladesh Med Res Counc Bull. 2013; 39(1):11-3. [DOI:10.3329/bmrcb.v39i1.15791] [PMID]
38. Sood A, Midha V, Mehta V, Sharma S, Mittal R, Thara A, et al. How sustained is sustained viral response in patients with hepatitis C virus infection? Indian J Gastroenterol. 2010;29(3): 112-5. [DOI:10.1007/s12664-010-0006-3]