Available online at www.jpsscientificpublications.com
Volume – 10; Issue - 1; Year – 2024; Page: 01 – 07
DOI: 10.22192/iajmr.2024.10.1.1

Indo – Asian Journal of Multidisciplinary Research (IAJMR)
ISSN: 2454-1370

In vitro STUDY FOR MIXED TRIPLE ANTIBIOTICS AGAINST LOCAL ISOLATE OF MULTIDRUG RESISTANCE *Staphylococcus aureus*

Zahraa Abd Al Kareem[1], Rabab, J. H. AL-Hasseny[1] and Abbas. K. Almansorri[2*]

[1]College of Food Science, AL-Qasim Green University, Iraq
[2]College of Biotechnology, AL-Qasim Green University, Iraq

Abstract

The purpose of this study was to investigate the effects of various antibiotics and evaluate their in vitro potency against multi-drug resistant *Staphylococcus aureus* when they were used singly and in triple combination. The methods included the Muller-Hinton in agar method was utilised for the Well diffusion technique. During the course of this research, the following antibiotics were chosen at random: rifampin, vancomycin, tetracycline, clindamycin, erythromycin, impenen, oxacillin, and trimethoprim/ sulfamethoxazole. It was previously characterised in our laboratory as Methicillin - Resistant *Staphylococcus aureus* - MRSA. The *Staphylococcus aureus* isolate that was used in this work was isolated and identified from skin that had been affected by an abscess. The results of this study included a comparison was made between the antibiotics used in triple combination and each antibiotic used on its own to determine the potencies of the antibiotics. The best results were recorded inhibitory zone of vancomycin, tetracycline, and clindamycin was 42 millimetres in diameter when compared to other results that were obtained with antibiotics alone. However, the results that were obtained on MRSA isolates ranged from 0 to 40 millimetres in diameter when various triple combinations (63 combinations) were used.

Key words: Methicillin, MRSA, *Staphylococcus aureus,* Triple combination and Antibiotics

1. Introduction

Methicillin Resistant *Staphylococcus aureus* (MRSA) is a common species of this genus and is easily identified by its ability to produce coagulase and hence clot human plasma (Baggett *et al.*, 2003). *Staphylococcal* infection can affect many sites and organs of the human body. Invasion of the skin cause impetigo, cellulitis. In the lungs abscesses and pneumonia are the result. Infection of the heart leads to endocarditis. Meningitis and abscess formation can be the result of infection to the central nervous system as well as, Keratitis can be the result of eye infection (Barrett *et al.*, 1968).

***Corresponding author:** Abbas. K. Almansorri
E.mail: dr.abbas66@biotech.uoqasim.edu.iq
Received: 16.01.2024; ***Accepted:*** 18.02.2024;
Published: 26.02.2024.

Because of the prevalence of Methicillin - Resistant *Staphylococcus aureus* (MRSA), which is responsible for the formation of bacterial keratitis, the treatment of these infections is made even more difficult by the fact that this bacterium is resistant to a variety of available medications. Since the year 2002, the United States has been responsible for the cultivation of the first three vancomycin-resistant MRSA strains (Al-Sahllawi, 2002). Additionally, the introduction of a new class of antibiotics has played a significant role in the modification of management strategies for the treatment of corneal infections. Despite this, there are growing patterns of resistance even to these novel types of antimicrobial drugs (Lodise *et al.*, 2008; Al-Fu'ady, 2010). It was the intention of this study to investigate the effects

of a number of different antibiotics and to evaluate their *in vitro* potency against multi-drug resistant *Staphylococcus aureus* when they were used singularly and when they were used in triple combination.

2. Materials and Methods

The Muller-Hinton in agar method was utilised for the Well diffusion technique. During the course of this research, the following antibiotics were chosen at random: rifampin, vancomycin, tetracycline, clindamycin, erythromycin, impenen, oxacillin, trimethoprim or sulfamethoxazole. It was previously characterised in our laboratory as Methicillin - Resistant *Staphylococcus aureus* (MRSA). The *Staphylococcus aureus* isolate that was used in this work was isolated and identified from skin that had been affected by an abscess.

Bacterial isolate

An isolated local strain of MRSA that has been thoroughly identified and characterised as being resistant to many treatments, having been isolated from a severe infection The identification of the isolate was carried out in accordance with the standard procedures in order to validate the consistency of its diagnostic properties (Rose and Poppens, 2008).

Evaluation of susceptibility to Antimicrobial agents

The Muller-Hinton agar, also known as Oxoid, was utilised in order to investigate the susceptibility of antibiotics through the Well diffusion test. Vancomycin, rifampin, tetracycline, trimethoprim-sulamethoxazole, erythromycin, clindamycin and impenem, and oxacillin were the antibiotics that were utilised in Hi-media. These antibiotics were recognised to be effective against the infection. The findings of the antibiotic susceptibility test were evaluated in accordance with CLSI, 2010, at the number seven. The triple combination approach involves combining antibiotics in accordance with the Table - l.

Table - 1: Triple Combination Method

Antibiotics		Erythromycin(E)	Clindamycin(Cd)	Impenem(Imp)	Trime.Thoprim-Sulamethoxazole (Tri\Sulf)	Tetracycline (Tet)	Rifampin(Rf)	Vancomycin (Va)	Oxacillin (Oxo		Antibiotics
		1	0.25	0.06	0.5/9.50	1	0.015	1	0.5	Con. (pog/L)	
Oxacillin (Oxi)	0.5	OXI-OXI- E	OXI - CD-OXI	OXI- oxI	OXI- OXI-TRI\SULF	OXI-TET-OXI	OXI-RF-OXI	OXI-VA-OXI	OXI	0.5	Oxacillin (Oxi)
Vancomyc VA		VA-VA- E	VA-VA-CD	VA- VA-IMP	VA- VA-TRI/SULF	VA-VA-TET	VA- VA-RF	VA	OXI VA- VA		Vancomycin (VA)
Rifarnpin RD	0.015	RF-R.F - E	RF-RF-CD	RF-RF-IMP	RF- RF-TRI/ SULF	RF-RF TET	RF	VA	oxI	0.015	Rifampin (RD
Tetracycline (TET)		TET-TET- E	TET-TET-CD	TET-TET- IMP	TET-TETTRI/SULF	TET	RF – TET-TET	VA – TET-TET	OXI-TET-TET		Tetracycline (TET)
Trimethoprim Sulamethoxazole (TR/Sulf)	0.5/ 9.5	TR]/SUF-TRI/SULF - E	CD-TRI/SULF-TRI/SULF	IMP-TRJ/SULF-TRI/SULF	TRI/SULF	TRI/SUI.F-TRI/SUI.F-TET	RF TRI/SULF-TRI/SULF	VA-TRI/SULF-TRI/SULF	OXI-TRI\SULF-TRI/SU L F	0.5/ 9.5	Trimethoprim Sulamethoxazole (TR/SULF)
Impenem (MP)	0.06	E - IMP-IMP	CD - IMP-IMP	IMP	-IMP-IMP-TRI/SULF	TET – IMP-IMP	RF-IMP-IMP	VA-IMP-IMP	OXI-IMP-IMP	0.06	Impenem (IMP)
Clindamycin (CD)	0.25	E -CD-CD	- CD	IMP -CD-CD	-CD- CD-TRI/SULF	TET – CD-CD	RF-CD-CD	VA-CD. CD	OXI-CD-CD	0.25	Clindamycin (CD)
Erythroanycin (E)	1	E	E- E-CD	E- E-lMP	E- E TR\ SULF	E- E TR/SU LF	E- E-RF	E- E-VA	OXL	1	Erythroanycin (E)

3. Results and Discussion

A comparison was made between the antibiotics used in triple combination and each antibiotic used on its own to determine the potencies of the antibiotics. The best inhibitory zone of vancomycin, tetracycline, and clindamycin was 42 millimetres in diameter when compared to other results that were obtained with antibiotics alone. However, the results that were obtained on MRSA isolates ranged from 0 to 40 millimetres in diameter when various triple combinations (63 combinations) were used. The microdilution method was also utilized to determine MRSA's reaction to antibiotics when administered in combination at varying concentrations, confirming the findings reported in this study. Figure 1 illustrates the inhibition zone diameter for each antibiotic alone. It shows that there was zero inhibition for trimethoprim, oxacillin, rifampin, and sulfomthaxcozol, indicating that the bacteria exhibited complete resistance in antibiotic media. However, the other results showed that the inhibition zones were 18, 30, 12, 22, and 18 mm for vancomycin (VA), tetracycline (TET), erythromycin (E), clindamycin (CD), and Impenen (IMP), respectively. Tetracycline appearance Oxacillin (potency) inhibition Zone for bacteria.

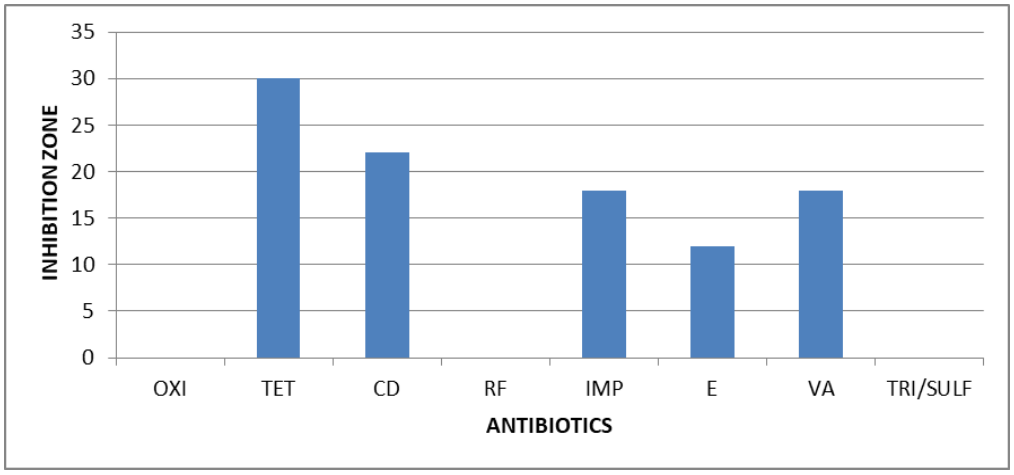

Figure - 1: The inhibition zone diameters of antibiotics alone

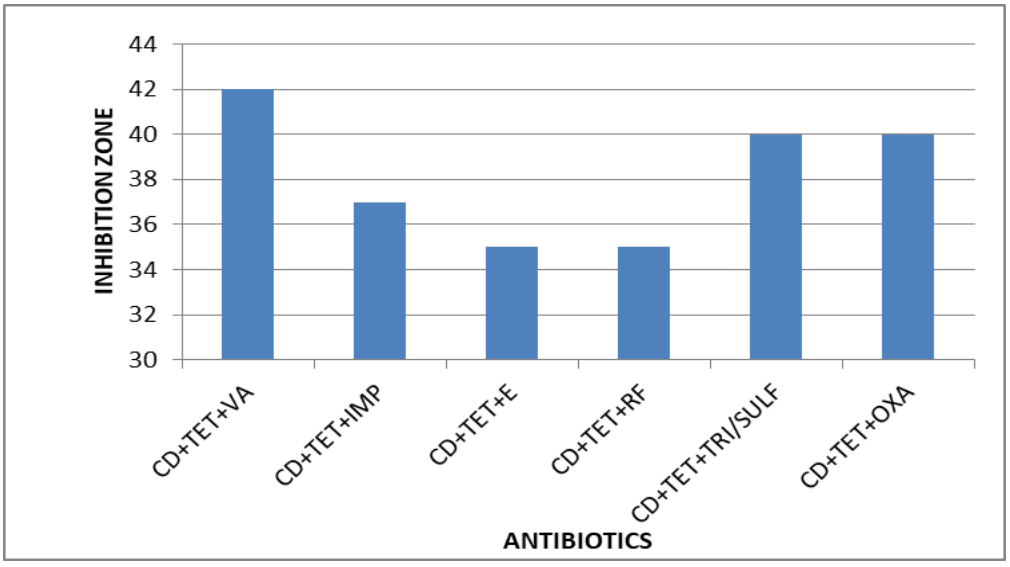

Figure – 2: The inhibition zone diameters of Clindamycin with other antibiotics

The strong result exhibited when combination of clindamycin (CD) with vancomycin (VA) and tetracycline (TET) as tribal mixed that inhibited 42 mm in diameter compare with other tribal combination of antibiotics that mixed with clindamycin. The result was acceptable in this experiment were observed in inhibition zone dimeter was 40 mm for each mixed CD with tetracycline with oxacillin or trimethoprim/sulfamethoxazole. And the likely IMP with CD when added of erythromycin, trimethoprim/sulfomethazole or Oxacillin as the show in Figure – 2. The tetracycline results were very excepted, when we ask combined the tetracycline with other antibiotics give potency result was tetracycline with clindamycin and trimethoprim/ sulfamethoxazole in present study can be explain in Figure - 3.

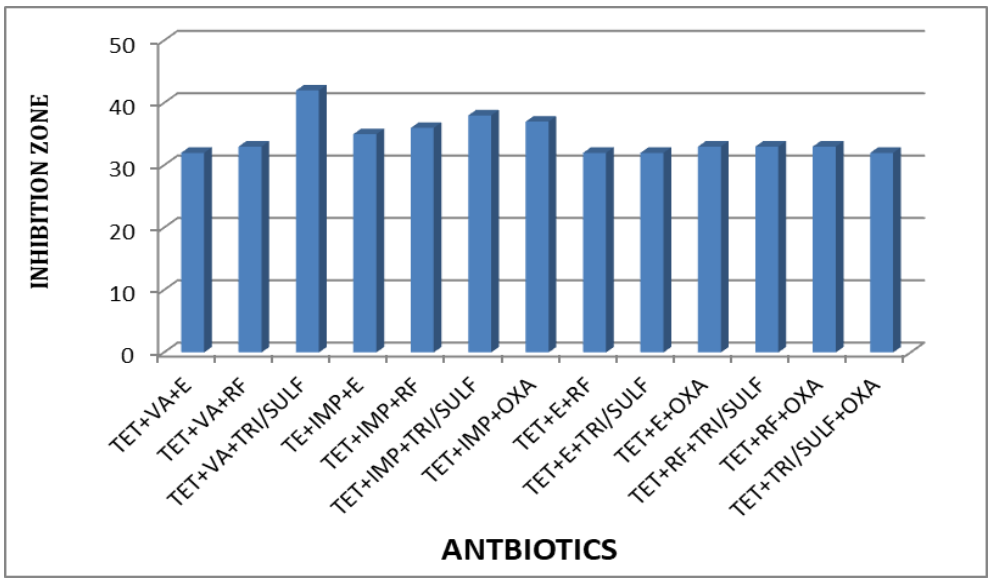

Figure – 3: The inhibition zone diameters of Tetracycline with other antibiotics

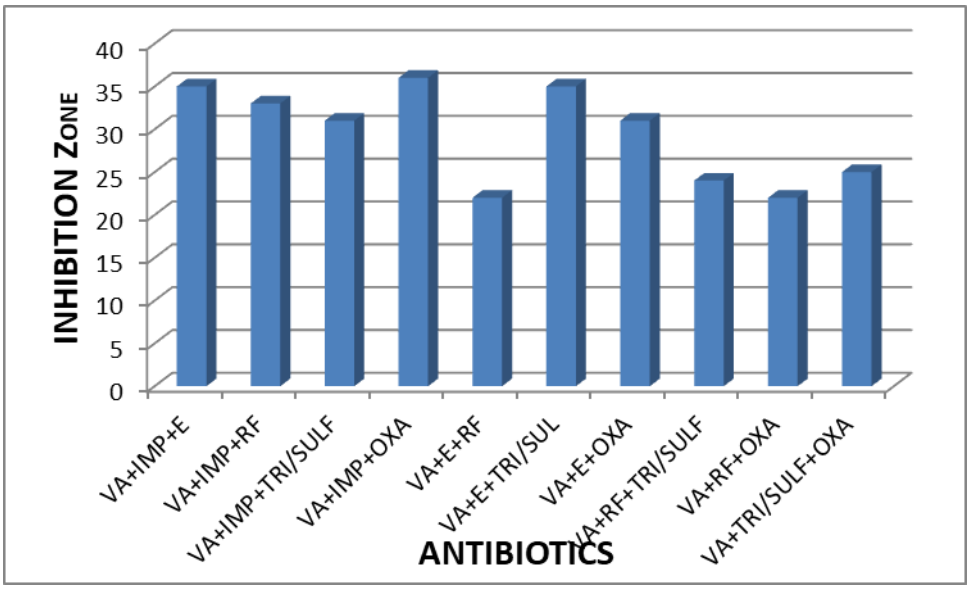

Figure – 4: The inhibition zone diameters of Vancomycin with other Antibiotics

In the Figure – 4, after mixed the vancomycin with other antibiotics 36 mm diameter was appearance around well combine when with Impenen and OXA but the inhibition zone dimeter was 35 mm for both mixed E &VA with IMP and TRI/ SULF & VA & IMP. These results were accordance with Nguyen and Grabber (2010). The response of MRSA infection to vancomycin often leads clinicians to add second antibiotics (CLSI, 2010), since vancomycin has been reported to be responsible on cell wall damage and warrant the second antibiotics to enter to the cell (Darouiche and Hamill, 1994). In addition the results in above the Impenen was effect on bacteria in present study. The inhibition zone was 35 mm appears when mixed the IMP with RF & OXA (Figure - 5).

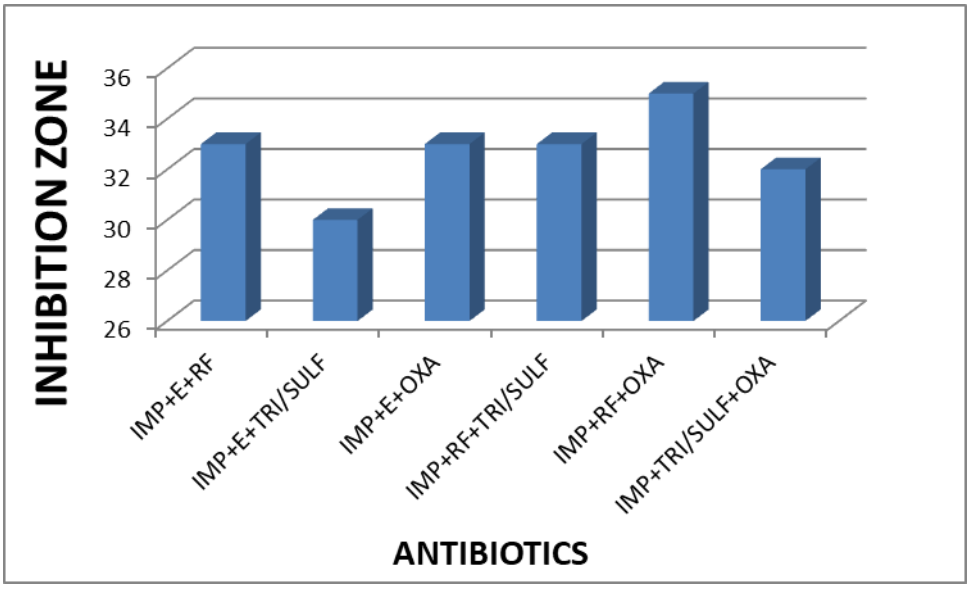

Figure – 5: The inhibition zone diameters of Impenen with other antibiotics

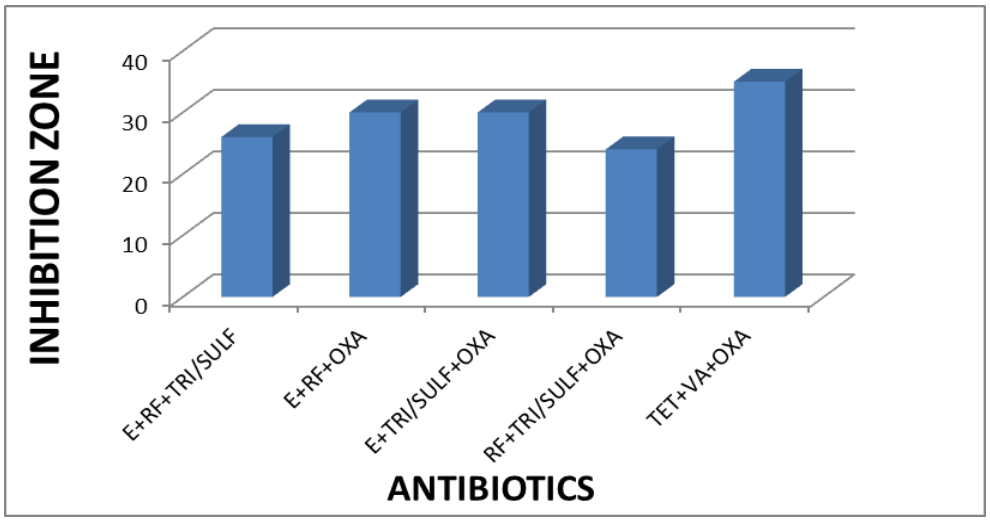

Figure – 6: The inhibition zone diameters of Erythromycin with other antibiotics

The Figure – 6 showed the results combination erythromycin with other antibiotics in present study. However, the erythromycin was antibacterial because it was given the diameter between 25 - 33 mm. On the light, the MRSA was explant resistance to antibiotics but when mixed tripled antibiotic in present study the MRSA was sensitive to antibiotics. The resistance of bacteria explained to carrying many virulence factors for example the bacteria have *SCCmec* gene included four type I, II, III and type IV. Four types have become wide spread (Deresinskics, 2009).

4. Conclusion

Synergisms of antibiotics were also seen, which represents the primary advantage of antimicrobial combinations. However, despite the fact that this study produced successful findings, it still takes use of distinct modes of action and/or toxicities profiles, as pointed out in the previous paragraph.

5. References

1) Baggett, H. C., Hennessy, T. W., Leman, R., Hamlin, C., Bruden, D., Reason over, A., Martinez, P., and Butler, J. C. (2003). An outbreak of community-onset Methicillin - Resistant *Staphylococcus aureus* skin infections in South-Western Alaska. *Infect. Hosp. Epidemiol.,* 24: 397 - 402.
2) Barrett, F. F., McGehee, R. F and Finland, M. (1968). Methicillin-resistant *Staphylococcus aureus* at Boston City hospital. *N. Engl. J. Med.,* 279: 441 - 448.
3) Al-Sahllawi, Z. S. R. (2002). A bacteriological study on local isolates of Methicillin Resistant *Staphylococcus aureus*. M. Sc. thesis, College of Science, University of Kufa.
4) Al-Fu'ady, A. H. (2010). Phenotypic and genotypic (*mec-A*) of Methicillin Resistant *Staphylococcus aureus* isolates in AL-Dewaniya. M.Sc. thesis. College of Medicine. University of Babylon.
5) Lodise, T. P., Graves, J. and Evanse, A. (2008). Relationship between vancomycin MIC and failure among patients with Methicillin - Resistant *Staphylococcus aureus* bacteremia treated with vancomycin. *Antimicrob. Agents Chemothe.,* 52: 3315 - 3320.
6) Baldoni, D., Haschke, M., Rajacic, Z., Zimmerli, W. and Trampuz, A. (2009). Linezolid alone or combined with Rifampin against Methicillin - Resistant *Staphylococcus aureus* in experimental foreign-body infection. *Antimicrob. Agents Chemothe.*, 3: 1142 - 1148.
7) Deresinski, S. (2009). Vancomycin in combination with other antibiotics for the treatment of serious Methicillin-Resistant *Staphylococcus aureus* infections. *CID*, 49: 1072 - 1079.
8) Rose, W. E. and Poppens, P. T. (2008). Impact of biofilm on the *in vitro* activity of vancomycin alone and in combination with tigecycline and rifampicin against *Staphylococcus aureus*. *J. Antimicrob. Chemother.,* 63: 485 - 488.
9) Clinical and Laboratory Standards Institute (CLSI). (2010). Performance standards for antimicrobial susceptibility testing. Approved standard M100- S17. Vol.27, No.1. NCCLS, Wayne, PA. USA.
10) Darouiche, R. O. and Hamill, R. J. (1994). Antibiotic penetration and bactericidal activity within endothelial cells. *Antimicrob. Agent Chemother.,* 38: 1059 - 1064.

Access this Article in Online

Quick Response Code	
Website	www.jpsscientificpublication.com
DOI Number	DOI: 10.22192/iajmr.2024.10.1.1
Thomson Reuters Researcher ID	K-4194-2016
ISI Impact Factor	3.652

How to Cite this Article:

Zahraa Abd Al Kareem, Rabab, J. H. AL-Hasseny and Abbas. K. Almansorri. (2024). *In vitro* Study for Mixed Triple Antibiotics against Local Isolate of Multidrug Resistance *Staphylococcus aureus. Indo-Asian Journal of Multidisciplinary Research*, 10(1): 01 – 07.

DOI: 10.22192/iajmr.2024.10.1.1