

# The Antimicrobial Effect Of +Oxivarea against Methicillin Resistance Staphylococcus Aureus and Pseudomonas Aeruginosa

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## Abstract

The World Health Organization recently published a list of bacteria which urgently needed new medicine; the examples of the bacterias are Methicillin Resistant Staphylococcus Aureus (MRSA) and P. aeruginosa. MRSA and P. aeruginosa are in a group which highly needed a new alternative potential medicine. MRSA and P. aeruginosa have progressively increased their resistance to antimicrobial drug, MRSA and P. aeruginosa are listed as Multi Drug Resistance Organism (MDRO) and Health Care Associated Infections (HAIs) bacteria, HAIs is still a major problem in both developed or developing countries around the world include many countries in Asia. The aim of this study was to find an alternative potential drug which is effectively killing MRSA. +Oxivarea is a substance combine of grape seed oil, olive oil, lemon oil, and nutmeg oil which has been through ozonized process. Ozone has a potential to emerge therapy and act as a potential antimicrobial. This in vitro experimental research used broth dilution test method with Mueller Hinton Agar as bacteria growing medium. The purpose of using this method is to observe the killing effect of +Oxivarea, determine the minimum ozone time. The gas chromatography test was performed to determine the substances of +Oxivarea. The experiment showed +Oxivarea with 4 hours ozonation process and in 100% concentration could totally eliminate MRSA and P. aeruginosa. The antimicrobial effect of the +Oxivarea was formed because of biochemical compound of +Oxivarea such as polyphenols, methyl palmitate, methyl oleat, methyl stearate, methyl linoleat and also because of the ozonation process effect the intact of bacteria cell wall. Our findings showed that +Oxivarea could kill MRSA and this knowledge is expected to be used for the beginning manufacturer of anti MDRO drug +Oxivarea.

## INTRODUCTION

Nosocomial infection is one of the main problem faced by hospitals in developed and developing countries across the world because the bacteria can mutate from origin due to continuous antibiotic therapy [1]. In a hospital especially hospitals in developing countries, hospital is an environment where health services are provide from medical staff to patients, and as a work environment for medical and other staff. There are changes in the colonization of microorganisms, pathogenic microorganisms carried by visitors from outside hospital and can infect patients treated at the hospital, whereas pathogenic organisms from the patient's body can enter the visitor's or health care personnel body's and brought to the environment where they live and carry out activities [2][3]. The World Health Organization recently published a list of bacteria which urgently needed a new medicine, MRSA and P. aeruginosa are two of the examples. MRSA and P.

aeruginosa are classified as Multi Drug Resistance Organism (MDRO) and Health Care Associated Infections (HAIs) bacteria. MDRO isolates have increased in recent years, and causing health concern on the society, carbamapenems is the most dependable drug of choices for MDRO, since resistance emerged, this problem leads to the discovery of anti MDRO drugs [9]. HAI is still a major problem that cause 99 thousand deaths in United State of America, and also patients have to spend more than 30 million dollars per year for therapies. According data from Centre for Disease Control in the United State at least 23.000 people die because of MDRO, and also 2 million of people are infected with MDRO [1]. Resistant gram positive cocci such as MRSA and resistant gram negative bacteria such as P. aeruginosa has increased the prevalencies of nosocomial infection about 50-60% [4]. Antibiotic resistance is a global concern and also effect more than 2 million people per year, a new approaches to kill MDRO are needed [1]. Natural

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herbal drugs have much more potential value to kill MDRO, +Oxivarea is made from two main oils combination, grape seed oil from *Vitis vinifera* and extra virgin olive oil from *Olea europaea*, combined with lemon distilled oil and nutmeg oil. +Oxivarea made through ozonated process, ozone therapy has been used by medical personnel since world war I but has suffered from lack of clinical findings and research. Our findings showed that +Oxivarea could kill MRSA and *P. Aeruginosa*, this knowledge is expected to be used for the beginning manufacturer of topical anti MDRO drug, +Oxivarea.

**MATERIAL AND METHODS**

This study was an in vitro experimental study performed in Microbiology Department Dr. Soetomo General Hospital, Surabaya, East Java, Indonesia. The in vitro study used clinically isolated MRSA and *P.aeruginosa* which were stored in microbank -800C at the department of microbiology of Dr. Soetomo General Hospital, and grown in nutrient broth at 370C for 24 hours followed by antimicrobial susceptibility testing using tube dilution method. Dilution antimicrobial sensitivity test method was started with preparing the test tube, all six tubes was filled with 1ml of +oxivarea with different concentration, first tube was filled with 100% concentration of +Oxivarea, second tube was filled with 50% concentration, third tube was filled with 25% concentration, fourth tube was filled with 12.5% concentration, fifth tube was filled with 6.25% concentration, sixth tube was filled with 3.125% concentration of +Oxivarea, and 1ml MRSA suspension was added in to all tubes, all steps are repeated for the *P.aeruginosa* sensitivity test. Minimum Bactericidal Concentration (MBC) were observed by streaked the suspension on each tube into a Mueller Hinton Agar plate, and incubated for 24 hours. After the incubation period end, bacterial growth on the plate can be observed.

**RESULTS**

Seen in the picture below, after 24 hours incubation process, MRSA still grow in 3,125% to 50% of +Oxivarea concentration, but in 100% concentration, +Oxivarea can totally eliminate the MRSA.+Oxivarea can inhibit *P. aeruginosa* in 3,125% to 50% concentration, but in 100% concentration +Oxivarea can also totally eliminate *P. aeruginosa*.

**Figure 1**

MRSA growth on agar plate showed that 100% concentration of +Oxivarea can totally eliminate MRSA.



**Figure 2**

*P.aeruginosa* growth on agar plate showed that 100% concentration of +Oxivarea can totally eliminate *P.aeruginosa*.



**Table 1**

MRSA and *P. aeruginosa* growth data summary

Concentration	3.125%	6.25%	12.5%	25%	50%	100%
Bacteria						
MRSA	++	++	+	+	+	-
<i>P.aeruginosa</i>	+	+	+	+	+	-

**DISCUSSION**

Nosocomial or Healthcare Associated Infection is devastating complication and a global problem that cause high mortality and also morbidity, nosocomial infection can also increase the period of stay in hospital and increase patient’s charges event more it can lead to lifelong patient’s

disability [5]. MRSA and *P. aeruginosa* are two of the greatest bacteria that cause nosocomial infection, and also those bacteria also listed as MDRO, the result of antibiotic susceptibility testing showed that +Oxivarea can totally eliminate MRSA and *P. aeruginosa*. +Oxivarea are made from grape seed oil and olive oil that has been through ozonation process. Ozone therapy has been known since World War I, but ozone therapy has not been patented and suffered from insufficient research [6]. Recently many researchers were very interested in ozonated oil trials, they found it to have so many good effects such as destroying microorganism like bacteria, fungus, and parasites, ozone can kill bacteria through several mechanism, ozone can attacks amino acid such as glycoprotein, glycolipids, and also can block the enzymatic process of bacteria cell, ozone has capability to degrades nucleic acid this several activity leads to increase the membrane permeability leading to kill the bacteria[6][7]. In 2018 Song et al in their study demonstred that ozonated water have strong antibacterial effect on MRSA, ozone can kill both gram positive and negative bacteria, but ozone is unsatble molecule that decays to oxygen and release single oxygen atom [8]. Aghaei et al in their research found that ozone must in the gaseous form to be more efective, and also proposed that the best base to hold the gas are oils base, oil during the ozonation process engage ozone in the form of stable ozone[7].

## CONCLUSION

+Oxivarea is a mixture of oil that has been through ozonation process, oils mixture is a great base to hold the gaseous form of ozone. In this in vitro trials showed +Oxivarea can inhibit MRSA and *P. Aeruginosa* to grow at 3,125% concentration to 50%, and effectively eliminate MRSA and *P. Aeruginosa* at 100% concentration. Further in vivo studies of +oxivarea are needed to investigate the potential for healing process on infected wound.

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