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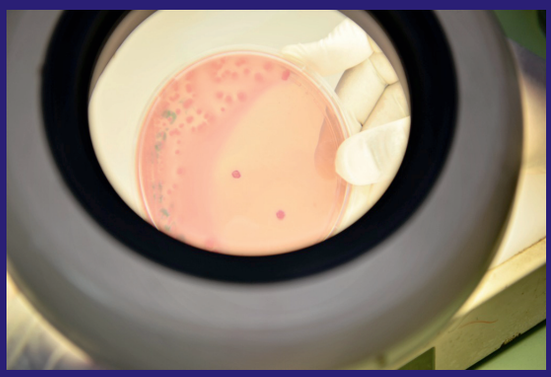




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# *Indonesian Journal of* Tropical and Infectious Disease

***CONTENTS***



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***Original Article***

# Long-term Consequences, Chances of Re-infection, and Outcomes among Cases Recovered with Severe COVID-19 at a Tertiary Care Centre in Central India

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***ABSTRACT***

*COVID-19 has a wide disease spectrum. Different presentations may be seen in different people, with uncertain long-term fate. The amount and longevity of immunity provided among the infected also vary from person to person which might in turn affect the chances of re-infection. Current study tries to uncover the incidence, disease severity and outcomes amongst those who have been previously hospitalized for COVID-19. A prospective cohort study where all patients admitted to intensive care facility at the tertiary care center were followed up for any occurrences of re-infection for more than one year. All cases were followed up telephonically and at scheduled visits to the hospital by trained personnel. A total of 410 cases with a mean age of 59.8 years, including 310 (75.6%) males and 100 (24.4%) females. Among these 410 patients 287 remained alive till the end of study period. Re-infection rates among recovered ICU admitted seriously ill patients were 1.4% whereas the rate of ICU re-admission due to COVID-19 re-infection was only 0.7%. Re-infection among female was 1.1% whereas in male was 1.5%. ICU readmission rate among female was 1.1% while in male was 0.5% only. The chances of re-infection in female were seen less than that in males, but the severity of re-infection in females was found to be higher. COVID-19 re-infection in previously severely infected COVID-19 patient is not so common. The chances of a severe disease among such cases are even rarer.*

***Keywords:*** *COVID-19; ICU patients; re-infection; SARS-CoV-2*

***ABSTRAK***

*COVID-19 memiliki spektrum penyakit yang luas. Presentasi yang berbeda dapat dilihat pada orang yang berbeda, dengan keadaan jangka panjang yang tidak pasti. Jumlah dan masa kekebalan yang diberikan di antara orang yang terinfeksi juga bervariasi dari orang ke orang yang pada gilirannya dapat mempengaruhi kemungkinan infeksi ulang. Studi saat ini mencoba mengungkap kejadian, tingkat keparahan penyakit, dan hasil di antara mereka yang sebelumnya dirawat di rumah sakit karena COVID-19. Sebuah studi kohort prospektif di mana semua pasien yang dirawat di fasilitas perawatan intensif di pusat perawatan tersier ditindaklanjuti untuk setiap kejadian infeksi ulang selama lebih dari satu tahun. Semua kasus ditindaklanjuti melalui telepon dan pada kunjungan terjadwal ke rumah sakit oleh personel terlatih. Sebanyak 410 kasus dengan usia rata-rata 59,8 tahun, termasuk 310 (75,6%) laki-laki dan 100 (24,4%) perempuan. Di antara 410 pasien 287 tetap hidup sampai akhir masa studi. Tingkat infeksi ulang di antara pasien yang dirawat di ICU yang pulih dan sakit parah adalah 1,4% sedangkan tingkat masuk kembali ke ICU karena infeksi ulang COVID-19 hanya 0,7%. Infeksi ulang pada wanita adalah 1,1% sedangkan pada pria adalah 1,5%.*

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*Tingkat penerimaan kembali ICU pada wanita adalah 1,1% sedangkan pada pria hanya 0,5%. Kemungkinan infeksi ulang pada wanita terlihat lebih kecil dibandingkan pria, tetapi tingkat keparahan infeksi ulang pada wanita ditemukan lebih tinggi. Infeksi ulang COVID-19 pada pasien COVID-19 yang sebelumnya terinfeksi parah tidak begitu umum. Kemungkinan penyakit parah di antara kasus-kasus seperti itu bahkan lebih jarang.*

***Kata kunci:*** *COVID-19; infeksi ulang; pasien ICU; SARS-CoV-2*

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## INTRODUCTION

COVID-19 often presents with an extensive clinical spectrum varying from asymptomatic infection to severe life-threatening viral pneumonia often requiring admission to intensive care, and sometimes even leading to death.[1] Persisting symptoms, and unforeseen organ dysfunction has been observed subsequently to SARS-CoV-2 infection in an escalating quantum of those who have recovered, as was observed in the past during SARS outbreak.[2] However, since COVID-19 is not a classical disease, we need to keep vigil about gaining new insights in it and an uncertainty prevails concerning its long-term health sequelae amongst those who have recovered from it. This is of immediate relevance and warrants attention since patients presenting with grave disease including those requiring mechanical ventilation during their initial medical admission, for whom long-term complications, persisting symptoms and sometimes who might lack a complete recovery on discharge.[3] This is an initial general concept that the patients who have recovered from COVID-19 natural infection generate a robust immune response which help in clearing the virus.

However, currently it is not very clear whether such primary exposure or disease confers a shielding immunity to successive infections with this virus. Recent studies suggest that antibodies generated after a recent COVID-19 infection might help in providing some protection against re-infection in most patients but despite this, re-infection or break-through infection is possible.[4] From previous research, it is clear that despite the presence of antibodies re-infection is common with other human corona viruses.[5] According to a recent report the working epidemiological case definition for re-infection after initial infection of COVID-19 was suggested as two positive tests at an interval of at least 102 days with one interim negative PCR test report.[6] Few case series show that recurring COVID-19 infections might be worse in approximately 20% of patients and even severe complications may occur among the higher those with advanced age and immune-compromised patients.[7] Re-infection with COVID-19 is not limited to any particular stain, there are multiple variants with a differing genetic sequence, thus causing re-infection.[8]

Subsequently, to the emergence of the newer mutants and variants of concern of COVID-19 from the UK, India and South Africa; It becomes indispensable to see whether these newer mutants cause any infection to patients who were affected with this disease during the 'first wave' prior to the appearance of these variants.[7] It is thought that as there is priming of adaptive immune response by the previous infection, re-infection is usually associated with milder symptoms, protection from severe disease but the robust response has also been reported.[9,10]

There might be numerous SARS-CoV-2 re-infection cases than have been currently reported.[11,12] It is very difficult to estimate the true prevalence of these re-infections as the genome sequencing data are not available in most COVID-19 cases and many of the asymptomatic and mildly symptomatic patients were not seeking medical advice. For

identification of true prevalence of COVID-19 re-infection population-based studies are more useful.

It might often be challenging to differentiate between COVID-19 relapse, re-infection and RT-PCR re-positivity in a few cases. Recently, Yahav et al had suggested that re-infection is considered in those case who become negative after infection and again became PCR positive after more than 90 days.[13] Two of the meta-analyses performed during early phase of the pandemic reported that re-infection or re-positivity were rare but such case reports and studies were performed without considering genome sequencing data.[14]

Few studies have shown that subsequent infection is possible in those persons already having a previous exposure to COVID-19. Therefore, practicing social distancing and wearing mask at all public places, irrespective of history of prior infection or vaccination is very essential to prevent the spread of further waves of the current pandemic. Without which, it's likely that the SARS-CoV-2 virus may continue to transmit and circulate in various populations despite the achievement of herd immunity by vaccination or natural infection.[15]

This study is concerned with the detection of re-infection if any, the associated disease severity and the outcome of such re-infection during the subsequent waves of COVID-19 infections among ICU hospitalized patients during the earliest wave of this disease.

## MATERIALS AND METHODS

### Research Design

This study consisted of cohorts enrolled prospectively at a Tertiary Care Center, in Central India, Madhya Pradesh. Study was started after due approval from institutional human ethical committee reference number, IECBMC/2021/32.

### Inclusion and Exclusion Criteria

Inclusion Criteria:

1. We included and followed up all critically ill ICU patients with laboratory-confirmed SARS-CoV-2 PCR positivity via recommended throat swabs or nasopharyngeal swabs,
2. Those who were discharged from the institute between August 2020 to November 2020 during the first wave of COVID-19.

Exclusion Criteria:

1. Those who refused to participate,
2. Those who died before the follow-up visit,
3. Those who could not be contacted.

All discharged patients met uniform discharge criteria according to the Government of India IMCR Guidelines for COVID-19.[16]

### Patient Follow-ups

Phone calls were used to schedule follow-up visits and done by trained medical staff. Post-discharge such patients were contacted in the order of their symptom onset date as per initial admission record. If the follow-up appointment was missed, 2 more chances on further dates were provided. Follow-up consultations were done with face-to-face interviews and examinations performed by trained medical personnel.

### Data Analysis

Data were abstracted and fed into computer on excel sheets, percentages and proportions were calculated using the same software.

## RESULTS AND DISCUSSION

During the study 410 cases in total between the ages of 1-month old to 95 years, and population having mean age 59.8 years. More than half i.e. 209 (51%) patients were between 51 to 70 years of age. The sex distribution of study cases was observed to have 310 (75.6%) males versus 100 (24.4%) females. Among 410 patients, 287 remained alive after first wave was over (shown in Table 1). Between first wave and second wave during the study, 5 persons died with the reasons behind their death remaining unclear and not directly related to COVID-19.

A single dose of vaccine was received by 196 (partially vaccinated) while 78 were vaccinated by both the doses of vaccination before second wave of COVID-19. Among these 287 individual only 4 were infected in the second wave of and only 2 were admitted in ICU. The two patients those who were re-admitted in ICU were partially vaccinated. Former being a 48 years old female and later one a 50-year-old male. The other two who were admitted in general ward were 32 years old male, he was fully vaccinated and other one was youngest patient 10 months old non-vaccinated male child, all four were discharged successfully. The mean age of those re-infected was 33±19 years and this study population comprising of 25% females and 75% males as shown in Figure 1. Only female patient was having hypertension as a co-morbidity.

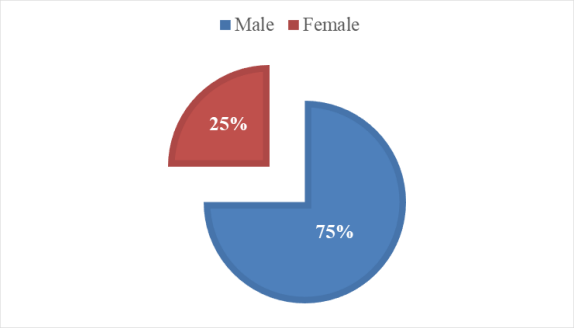


**Figure 1.** Sex Wise Distribution of Re-infected Patient

Re-infection rate among recovered ICU admitted seriously ill patients was 1.4% whereas the rate of ICU re-admission due to COVID re-infection was only 0.7%. Re-infection among female was 1.1% whereas in male was 1.5%. ICU readmission rate among female was 1.1% while in male was 0.5% only. The chances of re-infection in female were seen less than that in males, but the severity of re-infection in females is more was found to be higher.

**Table 1.** Age-Wise Distribution of Study Cohorts

| No | Age group (year) | Patients | | | Deaths | | |
|---|---|---|---|---|---|---|---|
| | | Male | Female | Total | Male | Female | Total |
| 1 | ≤ 30 | 18 | 7 | 25 | 2 | 1 | 3 |
| 2 | 31–40 | 34 | 8 | 42 | 6 | 2 | 8 |
| 3 | 41–50 | 58 | 16 | 74 | 8 | 3 | 11 |
| 4 | 51–60 | 70 | 33 | 103 | 23 | 6 | 29 |
| 5 | 61–70 | 86 | 20 | 106 | 32 | 9 | 41 |
| 6 | > 70 | 44 | 16 | 60 | 22 | 9 | 31 |

In a meta-analysis study by Ghorbani et al, the overall estimation of reinfection, was 3% (95% CI: 0.8–5), recurrence was 133 (95% CI: 105–160), with readmissions being 75 (95% CI: 54–96) per 1000 patients[17], but in our study rate of re-infection leading to hospitalization was only 0.7%. This is close to the study done by Arafkas et al. where the prevalence of re-infection was reported as zero.[18] Other study done by of Ren et al. reported a re-positivity of 12%, while Piri et al. concluded in their systematic review a recurrence rate between 2.3% to 21.4%.[19,20] In addition their review indicates that the recurrence was 47.7% in male and 53.3% in female which is in contrast to our study in

which recurrence was most common in male than female. In our study males were three times more affected than females.[20]

In some of the studies, re-infected, recurrent, and readmitted cases were either asymptomatic or had mild to moderate symptoms[17], but in our study all patients were symptomatic, some of them even had rare symptoms and complications like hepatitis which were rarely seen earlier.[21] This may be attributed to the reason that asymptomatic or patients with mild symptoms were not reported to near medical facilities and therefore they did not get tested for COVID 19. Few patients even had severe symptoms in the second phase of infections of the disease, implying that the severity of its subsequent infection may vary according to the demographics, health status of the patients, and immune system status.[22,23]

In the study incidence density per 100,000 person days was 1.0 (95%, CI 0.5–1.5) among persons having previous history of infection and 15.1 (95% CI, 14.5–15.7) for persons lacking such infection in the past.[24] Our findings are in agreement to the to those of Harvey and colleagues, who found that persons with a positive diagnostic RT-PCR test for SARS-CoV-2 and for antibodies to it were much less likely to develop SARS-CoV-2 infection within initial 3 months than those with absence of antibodies.[25]

## CONCLUSIONS

COVID-19 re-infection in previously severely infected COVID-19 patient is not so common. The chance of having a severe disease in these patients upon re-infection is even rarer. However, large scale population based elaborate case control study may be required in this field in order to provide further insights.

## ACKNOWLEDGEMENT

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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***Original Article***

# Relationship between Knowledge and Preventive Behavior of Leptospirosis in Berbah District Sleman Regency Yogyakarta 2021

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***ABSTRACT***

*Public Health is the resultant from an balance of individual, agent, and environment problems. Individual knowledge and the ability to adapt to the environment vary greatly. The individual awareness and sensibility towards surroundings will contribute to the public health status. Environmental factor in the raining season with high intensity of rainfall cause some risks such as flood. It can be a disaster and destroy the facilities in the area. This can be a transmission media, a new habitat of insects for certain disease, one of them is Leptospirosis. The condition after flood with low level of clean water facilities can make these bacteria live and reproduce, with warm temperatures, neutral pH of water, humidity and also high rainfall. Leptospirosis cases in Indonesia are sufficiently high which in 2019, there are 845 cases with mortality rate for 16.26%. Some factors may affect the number of the case. This research is aimed to know the factors related to the preventive behavior of leptospirosis. It was held in Public Health Center Berbah Yogyakarta. The method of research is quantitative research with design survey method, quasi experiment. The population is the clients visiting to Public Health Center Berbah, with the average number of clients coming are 200 clients/day. The sample is taken by accidental sampling to the clients with the number of daily visits (50 persons). The result shows, that of five analyzed factors, knowledge is the factor related to the preventive behavior of leptospirosis.*

***Keywords:*** *behavior; knowledge; leptospirosis; prevention; public health*

***ABSTRAK***

*Kesehatan masyarakat merupakan gabungan resultance dari keseimbangan masalah individu, agent, dan lingkungan. Pengetahuan individu, dan kemampuan untuk beradaptasi terhadap lingkungan sangat bervariasi. Kesadaran dan kepekaan individu terhadap lingkungan sekitar akan berkontribusi terhadap status kesehatan masyarakat. Faktor lingkungan saat musim penghujan dengan curah hujan yang cukup tinggi, berisiko menimbulkan banjir yang menjadikan suatu bencana dan dapat berakibat rusaknya sarana serta dan lingkungan sekitar. Hal ini dapat menjadi media penularan, menjadi sarang serangga/vektor suatu penyakit diantaranya penyakit leptospirosis. Kondisi pasca banjir dengan sarana air bersih yang kurang bagus, menyebabkan bakteri penyakit ini hidup dan berkembangkiak, suhu yang hangat, pH air yang netral, dan kelembaban serta curah hujan yang tinggi. Kejadian leptospirosis di Indonesia masih cukup tinggi, tahun 2019 tercatat 845 kasus dengan angka kematian 16,26%. Beberapa faktor mungkin berpengaruh terhadap angka kejadian leptospirosis. Penelitian ini bertujuan untuk mengetahui faktor-faktor yang berhubungan dengan perilaku pencegahan penyakit leptospirosis. Penelitian dilakukan di Puskesmas Berbah Yogyakarta tahun 2021. Metode penelitian adalah penelitian kuantitatif dengan menggunakan metode survey rancangan quasi experiment. Populasi adalah klien yang berkunjung ke Puskesmas Berbah, dengan rata-rata jumlah klien datang 200 klien/ hari.*

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*Sedangkan yang menjadi sampel adalah sejumlah klien yang diambil dengan cara accidental sampling, sejumlah klien dari jumlah rata-rata kunjungan setiap hari (50 orang). Hasil penelitian menunjukkan bahwa, dari kelima faktor yang dianalisis, pengetahuan adalah faktor yang berhubungan dengan perilaku pencegahan penyakit leptospirosis.*

***Kata kunci:*** *kesehatan masyarakat; leptospirosis; pencegahan; pengetahuan; perilaku*

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## INTRODUCTION

Indonesia is a tropical country that has two seasons, dry and rainy season. In early November to January, it is the raining season in which some areas are in flood. This condition brings some risk not only the disaster itself but also some diseases suffered such as diarrhea, skin disease, Acute Respiratory Syndrom (ARI), and leptospirosis. Leptospirosis is also known as post-flood cold. It is one of zoonosis disease which is caused by rats as the carrier chain. It was initially discovered by Weil in 1886, but in 1915, Inada found the causative bacteria which was Spirochaeta of the genus Leptospira.[1] Among the genus, only *Species Interrogans* are pathogenic to animals and humans which are fewer than 180 *Serotype* and 18 *Serogroup*. One kind of *serotypes* can generate to a differently clinical picture which in contrast a clinical picture for aseptic meningitis can be affected by *Serotype.* Leptospirosis has a widely and varieties clinical manifestation.

It is broadly distributed worldwide, especially in tropical and subtropical countries which have high rain intensity. Yet there has been no evidence to the number of leptospirosis cases in the world, but it can be expected that the case can happen in the areas with high risk factors for exposure to leptospirosis by more than 100 cases per 100,000 every year. In tropical country with high humidity, the number of cases can be ranged 10–100 for 100,000 citizens each year, while in subtropical country; it is around 0.1–1 per 1,000,000 as well. The mortality rate from leptospirosis in some places worldwide was reported about 5–30%. These numbers are not significantly reliable because there are still a lot of areas in the world with properly undiagnosed leptospirosis. Cases in Indonesia are moderately high which in 2019 there are 845 cases by 16.26% mortality rates.

In 1970–2012, there were about 318 outbreak cases with the average of 7 outbreaks per year. These areas are Latin America and Caribbean Island (36%), followed by Southern Asia (13%) and Northern America (11%). Most outbreak cases happened in tropic and subtropical areas (55%). The risk factors that can cause the outbreak are outdoor activities (25%), exposed to flood water (23%) and puddle (22%).[2] Other significantly risk factors are living in rubber plantation and taking a bath in a natural bath.[3] The burden caused by leptospirosis is fairly high but there is less accurate diagnosis due to the lack of public awareness to this disease. World Health Organization (WHO) and Leptospirosis Burden Epidemiology Reference Group (LERG) hold the evaluation of leptospirosis burden and transmission. Some risk factors are found as the causes of leptospirosis such as water exposure, recreational water/swimming activities in developing countries, flooding and heavy rain.[4] While other studies states that the risk factors in public are the environment close to the river, garbage, poor sanitation and many rats around the house. In this case, society has tried some prevention to the spread of the disease.[5]

Looking at the phenomena, the awareness is required towards leptospirosis and epidemiology development. Confirming cases that are spreading and epidemiological

knowledge of leptospirosis such as life cycles, geographic patterns, populations most at risk that are needed for adequate prevention of the spread of leptospirosis, one of which is preventive knowledge of the disease.[6] This research is aimed to know the factors related to the preventive behavior of leptospirosis.

## MATERIALS AND METHODS

The type of research is quantitative research with a design survey method, using an accidental sampling technique. The research was conducted in November 2021 where the frequency of rain is still very high. The community in the Berbah Health Center area has never received counseling related to leptospirosis and most of the community as farmers, they do not use footwear when farming in the fields. We received ethical clearance from Stikes Surya Global with number 210/KEPK/SSG/I/2022. The population is the clients visiting to Public Health Center Berbah, with the average number of clients coming are 200 clients/day. The sample is taken by accidental sampling to the clients with the number of daily visits in Public Health Center Berbah. Analysis using ANOVA. Questionnaire was used in this research are knowledge about leptospirosis questionnaire and behavior questionnaire. After processing the data with the frequency distribution, the following categorical data is obtained. The categorization of knowledge in this research divided into 3 categorization which good (76%–100%), adequate (56%–75%) and less (<56%). For behavior, it is divided into 2 categorization which good (≥15) and poor (<15).[7]

## RESULTS AND DISCUSSION

The result of the research, related to characteristic frequency distribution, can be described that there are 27 females and 23 males as the respondents whom 14 of them working as farmers. Leptospirosis case is appeared due to not only the factor of knowledge but also their occupation as farmers. The structured interview was collected from mostly famers and this job has a direct contact to dirty water, place, garbage and animal. If no one uses personal protective equipment and there is an injury, Leptospira bacteria can enter through wounds and humans can get leptospirosis.

**Table 1.** Respondent Characteristic Distribution

| Variable | Category | Total (n=50) | Precentage (%) |
|---|---|---|---|
| Gender | Female | 27 | 54 |
| | Male | 23 | 46 |
| Age | 10-20 years old | 2 | 4 |
| | 20-30 years old | 7 | 14 |
| | 31-40 years old | 8 | 16 |
| | 41-50 years old | 5 | 10 |
| | 51-60 years old | 18 | 36 |
| Occupation | Jobless | 9 | 18 |
| | Government employee | 1 | 2 |
| | Farmer | 14 | 28 |
| | Private employee | 6 | 12 |
| | Seller | 9 | 18 |
| Education | No Education | 1 | 2 |
| | Elementary School | 9 | 18.0 |
| | Junior High School | 14 | 28.0 |
| | Senior High School | 18 | 36.0 |
| | University | 8 | 16.0 |
| Knowledge | Good | 16 | 32 |
| | Adequate | 10 | 20 |
| | Less | 24 | 48 |
| Preventive Behavior | Good | 19 | 38 |
| | Poor | 31 | 62 |

Table 1 shows the characteristics of the respondents, more than a third of the respondents were elderly (51–60 years), worked as farmers, had a high school education and almost half had very low knowledge and prevention behaviors.

**Table 2.** Table of Homogenic Test to the Factors Related to the Behavior

| Variable | Levene statistics | Significancy | Interpretation |
|---|---|---|---|
| Age | 3.024 | 0.014 | Not homogene |
| Gender | 0.533 | 0.469 | Homogene |
| Education | 0.323 | 0.808 | Homogene |
| Job | 0.864 | 0.493 | Homogene |
| Knowledge | 2.184 | 0.037 | Not homogene |

Table 2 shows the results of the homogeneity test for the five variables, where two of the five were declared not homogeneous.

**Table 3.** Model Summary

| Model | R | R Square | Adjusted R Square | Std. Error of the Estimate |
|---|---|---|---|---|
| 1 | 0.506[a] | 0.256 | 0.240 | 2.949 |

Table 3 explains that the magnitude of the correlation value is 0.506. From the outputs, it can be obtained that the coefficient of determination (R Square) is 0.256 which means that the influence of knowledge variables on behavioral variables is 25.6%.

**Table 4.** ANOVA Test

| Model | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Regression | 143.602 | 1 | 143.602 | 16.515 | 0.000[b] |
| Residual | 417.378 | 48 | 8.695 | | |
| Total | 560.980 | 49 | | | |

Table 4 shows that F value is accounted 16.515 with the significant level as much as 0.000 < 0.05 therefore it can be concluded that there is a relationship between knowledge and the society behavior towards Leptospirosis in Berbah District, Sleman Regency, Yogyakarta. From the results above, it can be drawn that knowledge also affects towards the behavior to Leptospirosis in the same area.

The incidence of leptospirosis is common in tropical countries, such as Indonesia and other Southeast Asian countries. One of the phenomena is that there is a high level of awareness on how to prevent this disease but the behavior toward its prevention still needs enhancement. This likely happened in Thailand where the society has the high awareness but the level of preventive behavior has not been optimal even though there is no leptospirosis recorded in this area. Thus, the perception and preventive behavior to Leptospirosis need to be continuously done and get the supports from various parties even though there is no Leptospirosis detected in certain areas. This can be done in ruder to have a good-behavior society towards the prevention of the disease.[8]

Leptospirosis is a disease caused by an interaction among factors such as human's activities, carrier animals, and environment. Domestic and wild animals can be the carriers of leptospirosis bacteria through their urine. Puddle when it is raining season can be a potential place of the leptospirosis transmission media to human. Transmission that allows the entry of these bacteria is through open wound that is exposed by water contaminated by leptospirosis bacteria.[9]

Berbah District, an area that stretches from north to south at the very end and has many very large rice fields, can still be found rats. Most of the respondents said that they often see rats around their houses from rice field areas. People living in Berbah District have a sufficient knowledge and a good behavior related to this disease. New information about something can give a new cognitive foundation in forming knowledge. This is corresponded to the theory from L. Green stating that someone's behavior towards their health is affected not only from their knowledge but also their beliefs, cultures, traditions, and so on.[10] Leptospirosis is still common, so in order to minimize the spread of the disease, some efforts should be taken

as an alternative approach which is *OneHealth* Approach. It is a combination of several sectors in handling some aspects regarding to the disease which those aspects are in the different sectors which are sector in handling humans, animals, and environment.[10]

Counseling will increase the respondent's knowledge and attitude, as it was held by Pujiyanti and Trapsilowati,[12] by the plan of *one group pre-post design* in Sedayu and Wukirsari Village, Bantul Regency. The result shows that there was a distinctive significant ($p<0.05$). As well as the significant influence, knowledge can be obtained from various sources such as counseling by giving information or knowledge. The factors affecting the knowledge are education, information/mass media, occupation, environment, experience, age, social, culture and economy. Public knowledge about leptospirosis can be influenced by a lot of factors. One of them is information from printed and electronic media as well. There is new information about something that can give a new cognitive foundation in forming knowledge towards a new perspective.[10]

A prevention towards a leptospirosis case is through the information distribution that also one of the ways to decrease the number of the leptospirosis case. The result from this method is a decent attitude and knowledge towards the attitude or preventive effort to leptospirosis. Similar to the research held in Medan showing that there were an adequate raise of the knowledge after giving a preventive information.[13] Beside that, education can also level up the awareness of the preventive behavior mainly if it can be done in a small group. The education got by the society about this disease is proven to be able to increase the number of society awareness to prevent the disease.[14]

The information about leptospirosis, besides being collected from public counseling, also has a source of information gathered from family/friend. Similar to the research from Pujiyanti et al[15] stating that the society was still in low level of knowledge and 80% of the society had a preventive behavior which was using the personal protection equipment when handling carcass and controlling rats.[15]

In this research, respondents' occupation is mostly farmers. The analysis shows that occupation does not have any relationship to the preventive behavior. The same result is also reported in some research, there was no correlation between occupation and the case of leptospirosis.[16,17]

Farmers are the jobs closely related to humid and wet conditions where they can be the place to get contaminated by leptospirosis bacteria. The jobs that have high risk to get infected are farmers,[18] vet, garbage collector, gutter cleaner, miner, or any other jobs that always have contact with dirty places. Moreover, when there is no sufficient self-protector. Maharani's research also stated that the majority of respondents, in the study of preventing a disease, had a high risk job and poor equipped. Therefore, the use of self-protectors such as gloves and boots has a role in preventing Leptospirosis disease. Thus, it requires enhancement to the awareness level to the use of self-protectors for risky jobs.[17] Not only directly contacted jobs that can affect the spread, but also the use of footwear could be one of the other factors. It is related to more than 50% respondents, in Samekto et al[19] study, who have not got any counseling yet. For this case, there should be a raise of the knowledge to each individual who works in scope of work at risk for leptospirosis.[18] Although working as a farmer is not related to the preventive behavior to the disease, but there should be any socialization to them as a preventive way. It is because the disease can be indirectly spread by the infected animal urine. Bacteria can survive for months in puddle and humid underground. Knowing that farmers are very close to this kind of environment then the preventive effort is required as well as the socialization to the preventive behavior of Leptospirosis.[20]

Age factor in this research has no relationship to the preventive behavior. The result is different to Hasanah, Nugraheni and Wahyuni's.[21] stating that there was a correlation between age and the behavior. Age is connected by the level of individual understanding in accepting information. The older the people, the more capable they can comprehend on what action or behavior that belongs to preventive actions. But in this research, age does not become a predictor to the self-awareness to prevent disease. It can be caused by some other factors such as their education background that is mostly dominated by high school level.

From this study, it can be obtained that knowledge is the only factor relating to the behavior to Leptospirosis.[21] A lot of factors can affect one's knowledge. These also contribute to decision and attitude in processing information, analyze, and apply something that has never been known before. Therefore, knowledge is very essential and a decisive value before health behavior works.[21,23]

Prevention is the way that can be used to reduce the spread of leptospirosis. It is by increasing the knowledge and behavior about leptospirosis.[24,25] Kipper et al[5] stated that some people had adequate knowledge this disease such as the transmission, symptoms, and prevention in a basic level. They also tried to stop the spread even though they has not completely understood of the spreading system, the host that carries leptospirosis and the factors affecting the big number of spreading in a community.[5]

Same theory was highlighted in Fadlilah's study stating that there is a relationship between knowledge and preventive practice to leptospirosis. It shows that the higher the level of education they have, the better the preventive behavior they do even if it is compared to the lower education level. From here, it also can be noticed that knowledge has a significant impact in increasing individual awareness of the preventive behavior to leptospirosis. Knowledge that they have will strengthen them in making decision. They who have a good knowledge and realize the danger of the disease will level up their awareness to do a preventive action.[22]

Limitations in this research were carried out while still in the COVID 19 pandemic, so it was necessary to limit contact with respondents. The behavior of society in this research majority is in the poor group. As the spreadness of leptospirosis, it is caused by the spread of the virus through open wounds. So it is closely related to how human behavior. Clean human behavior will be able to reduce the spread of leptospirosis infection. And vice versa, human behavior with low sanitation will increase the spread of infection.[26] Public health behavior is also an effort to improve environmental sanitation. One of the recognizable indicators of this environmental sanitation is the presence of rats. Good environmental sanitation results from healthy community behavior will reduce the number of rat species in the environment. This of course can prevent the further spread of leptospirosis.[27]

## CONCLUSIONS

From this research, it can be concluded that the individual's knowledge is related to the preventive behavior of Leptospirosis in Berbah District, Sleman, Yogyakarta.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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***Original Article***

# Characteristics of Leptospirosis Cases in Pacitan District, East Java Province

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***ABSTRACT***

*Leptospirosis is a disease that is still a public health problem in the world, however, these cases are rarely reported due to the difficulty of distinguishing clinical symptoms from other endemic diseases and the lack of appropriate laboratory diagnostic services. Pacitan district is one of the districts in East Java that reported Leptospirosis cases for 3 consecutive years from 2017 to 2019. There were total 92 Leptospirosis cases with Case Fatality Rate (CFR) of 15.22% in Pacitan. This study is a descriptive study with a Cross Sectional design that aims to describe the distribution of characteristics of Leptospirosis cases in Pacitan district based on person, place, and time. This study used secondary data from the Pacitan district Health Office, East Java province. The population in this study was all cases with Leptospirosis cases as many as 92 cases recorded in the Pacitan district Health Office data for 2017–2019. The sample of this study were all cases with Leptospirosis as many as 92 cases.The results of the study obtained Leptospirosis cases in Pacitan district in 2017–2019 based on person occured most in the age group of 40–49 years old by 20.45%, in the male sex by 68.48%, and in the population who worked as farmers by 73.58%. Based on the place where the most occured in Tulakan sub district by 52.75%, while based on time, most occured in February, March and April, this is because February to April is the rainy season. Therefore, based on the results of the study, it is necessary to educate the public, especially at risk groups, about the risk factors and Prevention of Leptospirosis.*

***Keywords:*** *leptospirosis; Pacitan; person; place; time*

***ABSTRAK***

*Leptospirosis merupakan salah satu penyakit yang masih menjadi masalah kesehatan masayarakat di dunia, namun kasus ini jarang terlaporkan karena sulitnya membedakan gejala klinis dengan penyakit endemik lainnya dan kurangnya pelayanan diagnostik laboratorium yang tepat. Kabupaten Pacitan merupakan salah satu Kabupaten di Jawa Timur yang melaporkan adanya kasus Leptospirosis selama 3 tahun berturut-turut dari tahun 2017 hingga tahun 2019. Terdapat 92 kasus Leptospirosis dengan Case Fatality Rate (CFR) sebesar 15.22% di Kabupaten. Penelitian ini merupakan penelitian deskriptif dengan desain Cross Sectional yang bertujuan untuk menggambarkan distribusi karakteristik kasus Leptospirosis di Kabupaten Pacitan berdasarkan orang, tempat, dan waktu. Penelitian ini menggunakan data sekunder yang diperoleh dari Dinas Kesehatan Kabupaten Pacitan, Provinsi Jawa Timur. Populasi dalam penelitian ini adalah seluruh kasus Leptospirosis sebanyak 92 kasus yang tercatat dalam data Dinas Kesehatan Kabupaten Pacitan tahun 2017–2019. Sampel penelitian ini adalah seluruh kasus Leptospirosis sebanyak 92 kasus. Hasil penelitian diperoleh kasus*

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*Leptospirosis di Kabupaten Pacitan pada tahun 2017–2019 berdasarkan orang paling banyak terjadi pada kelompok usia 40–49 tahun sebesar 20,45%, pada jenis kelamin laki-laki sebesar 68,48%, dan pada penduduk yang bekerja sebagai petani sebesar 73,58%. Berdasarkan tempat paling banyak terjadi pada Kecamatan Tulakan sebesar 52,75%, sedangkan berdasarkan waktu paling banyak terjadi pada bulan Februari, Maret dan April, hal ini karena pada bulan Februari hingga April merupakan musim penghujan. Oleh karena itu berdasarkan hasil penelitian, perlu dilakukan edukasi untuk masyarakat terutama pada kelompok berisiko mengenai faktor risiko dan pencegahan penyakit Leptospirosis.*

***Kata kunci:*** *leptospirosi; orang; Pacitan; tempat; waktu*

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## INTRODUCTION

Leptospirosis is a zoonotic disease that is a public health problem in the world. Leptospirosis is common in tropical and subtropical developing countries and has high rainfall.[1,2] The occurrence of Leptospirosis is not only related to climate and environmental conditions, but due to contact with environments contaminated with *Leptospira* bacteria such as agriculture, poor housing and waste disposal that can cause a source of infection. While in temperate countries, Leptospirosis can occur locally and can also be transmitted by people who come from abroad, especially those who visit the tropics.[2–4]

Annual incidence worldwide is estimated at >1 million cases, including approximately 59,000 deaths. The regions with the highest estimates of morbidity include South and Southeast Asia, Oceania, the Caribbean, parts of sub-Saharan Africa and parts of Latin America. Outbreaks can occur after heavy rains or floods in endemic areas, especially in urban areas in developing countries, where housing and sanitary conditions are poor. Leptospirosis outbreaks have occurred in the United States after floods in Hawaii, Florida and Puerto Rico.[5]

The incidence of Leptospirosis in subtropical countries is estimated at between 0.1–1 cases/100,000 inhabitants per year, while in tropical countries it is estimated at 10 cases/100,000 inhabitants per year and may increase to 100 cases/100,000 inhabitants in the event of an outbreak.[2] In the United States, an estimated 100–200 cases of Leptospirosis are identified each year, of which 50% occur in Hawaii.[1]

Cases of Leptospirosis in humans are generally reported from India, Indonesia, Thailand and Sri Lanka during the rainy season. Indonesia is a tropical country and some areas in Indonesia are endemic areas of Leptospirosis. Leptospirosis can be a public health threat in the event of extraordinary events, this is because in Indonesia there are several risk factors that affect the incidence of Leptospirosis such as the high population of rats (rodent) as a reservoir of Leptospirosis, poor environmental sanitation and flood areas are increasingly widespread.[6]

Leptospirosis is a rarely reported disease, one of the causes of which is the difficulty of distinguishing clinical symptoms from other endemic diseases and the lack of appropriate laboratory diagnostic services.[3,4] Leptospirosis cases in Indonesia are also not widely reported, only 9 provinces that report cases of Leptospirosis are DKI Jakarta, West Java, Central Java, Yogyakarta, East Java, Banten, North Kalimantan, South Sulawesi and Maluku.[7]

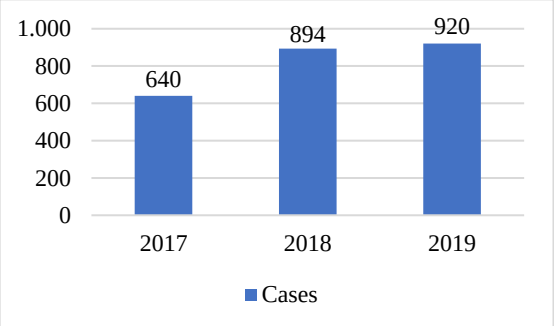


**Figure 1.** The Situation of Leptospirosis in Indonesia in 2017–2019[8]

The Figure 1 is showed an increase in Leptospirosis cases in Indonesia from 2017 until 2019 and there is a decrease in the case fatality rate from 2017 until 2019. The highest Leptospirosis cases occurred in 2019 and the lowest Leptospirosis cases occurred in 2017, while the highest case fatality rate occurred in 2017 and the lowest case fatality rate occurred in 2019. In 2017 there were 640 cases of Leptospirosis with a CFR of 16.88%, in 2018 there were 894 cases of Leptospirosis with a CFR of 16.55%, and in 2019 there were 920 cases of Leptospirosis with a CFR of 13.26%.[7]

East Java was one of the provinces that reported cases of Leptospirosis from 2017 to 2019, which consisted of 106 cases in 2017 with a CFR of 17.92%, in 2018 as many as 128 cases with a CFR of 7.81% and in 2019 there were 147 cases with a CFR of 15.65%.

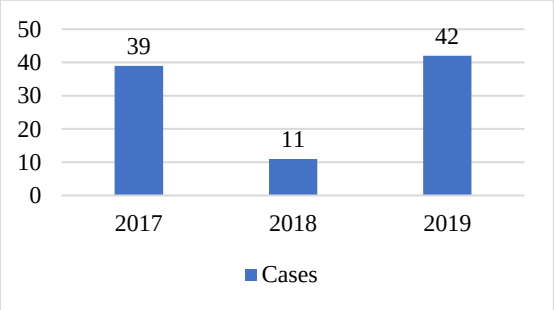


**Figure 2**. The Situation of Leptospirosis in Pacitan District in 2017–2019[8]

Pacitan district is an endemic area of Leptospirosis in East Java province, the Figure 2 is showed that Leptospirosis cases and case fatality rate fluctuate. In 2017, there were 39 cases of Leptospirosis and 32 cases of Leptospirosis recovered with a CFR of 17.95%, in 2018 there were 11 cases of Leptospirosis and 9 cases of Leptospirosis recovered with a CFR of 18.18%, in 2019 there were 42 cases of Leptospirosis and 27 cases of Leptospirosis recovered with a CFR of 11.90%.[8]

This study aims to describe the characteristics of Leptospirosis cases in Pacitan district, East Java province in 2017–2019 based on person, place, and time.

## MATERIALS AND METHODS

This study was a *descriptive* study with Cross Sectional design to describe the distribution of characteristics of Leptospirosis cases Pacitan district based on person, place, and time. The population and sample in this study were all cases with Leptospirosis with (92 cases) reported in the Pacitan district Health Office in 2017 until 2019. The data used in this study were secondary data obtained from the Pacitan district Health Office.

## RESULTS AND DISCUSSION

### A. Person

1. Sex

Based on Figure 3, the distribution of Leptospirosis cases by sex in Pacitan district in 2017–2019 is more common in men, namely 63 cases (68.48%), while in women as many as 30 cases (31.52%).

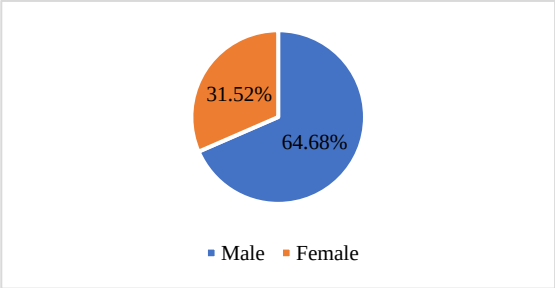


**Figure 3.** Distribution of Leptospirosis Cases by Sex in Pacitan District in 2017–2019[8]

In this study, it is stated that Leptospriosis cases that occurred in Pacitan district in 2017–2019 were more common in men, namely as many as 63 cases (68.48%). This study is in line with research conducted Prihantoro et al[9], 80% of Leptospirosis cases are male.[9] This study is also in line with research conducted in Boyolali, Central Java, Leptospirosis cases occur most in men by 70%.[10] Men are 37.01 times more likely to be infected with Leptospirosis than women.[11] This can happen because men have jobs that are more often exposed to environments contaminated with *Leptospira* bacteria.[12]

2. Age

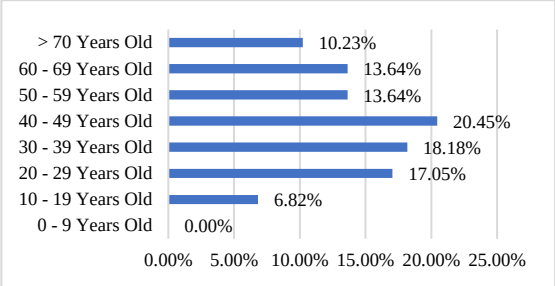


**Figure 4.** Distribution of Leptospirosis Cases by Age Group in Pacitan District in 2017–2019[8]

Based on Figure 4 is showed that the number of Leptospirosis cases in Pacitan district in 2017–2019 was most prevalent in the age group of 40–49 years old by 20.45%, in the age group of 30-39 years old by 18.18%, then in the age group of 20–29 years old by 17.05%, age group 50–59 and age 60–69 years old by 13.64%, in the age group >70 years old by 10.23%, and in the age group 10–19 years old by 6.82%, while in the age group 0–9 years old no cases.

In the research that has been done, it is stated that Leptospriosis cases that occurred in Pacitan district in 2017–2019 occurred most in the age group of 40–49 years by 20%. While in the age group <10 years there are no reported cases of Leptospirosis. Cases of Leptospirosis in children are rarely reported due to undiagnosed or different clinical manifestations with adults.[6] This study is in line with that conducted by Prihantoro et al[9], Leptospirosis cases occur at the age of more than 40 years old as many as 70 %.[9] Suprapto et al[13] said that the most cases of Leptospirosis in productive age (46–60 years).[13] This can happen because men in productive age tend to do more activities outside the home.

3. Job

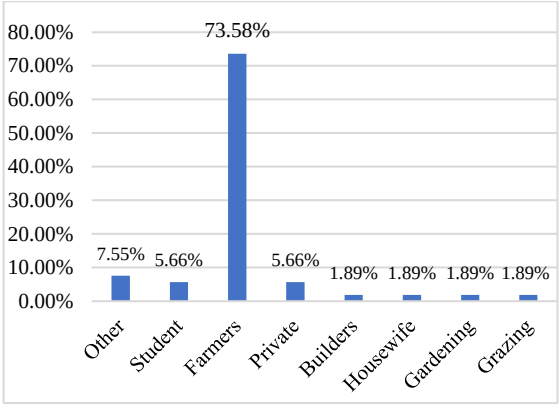


**Figure 5.** Distribution of Leptospirosis Cases by Jobs in Pacitan District in 2017–2019[8]

Based on Figure 5, Leptospirosis cases in Pacitan district in 2017–2019 occurred most in people who worked as farmers (73.58%), while in students, private sector and workers Leptospirosis cases amounted to 5.66%. In housewives, gardening and grazing the number of cases of Leptospirosis amounted to 1.89% and others to 7.55%.

Job is one of the risk factors for the occurrence of Leptospirosis. People who work in environments that contaminated with *Leptospira* bacteria are at risk of developing Leptospirosis.[6] The risk of Leptospirosis is higher in people who work outdoors or in contact with animals, such as farmers, planters, ranchers, slaughterers, veterinarians, veterinary nurses, mine workers, laboratory workers, fishermen, soldiers, fish traders, and traders in markets.[14,15,16]

Residents of rural areas who work as farmers and ranchers are at risk of contracting Leptospirosis.[6] This study showed that Leptospriosis cases that occurred in Pacitan district in 2017–2019 were the most common in cases who worked as farmers, namely 73%. This study is in line with research conducted Nuraini et al[10], 44.7% of cases with Leptsopsirosis occurs most in farmers.[10]

Raharjo et al[17] said that risky jobs have a 6,317 times higher risk of developing Leptospirosis than non-risky jobs.[17] While working as a farmer 2 times higher risk of Leptospirosis.[18] Samekto et al[19] stated that the habit of not wearing footwear is 4 times higher risk of developing Leptospirosis.[19]

## B. Place

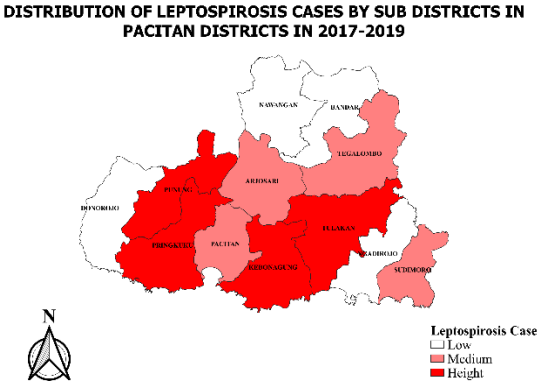


**Figure 6.** Distribution of Leptospirosis Cases by Sub Districts in Pacitan District in 2017–2019[8]

Based on Figure 6, the most Leptospirosis cases in Pacitan district in 2017–2019 occurred in Tulakan sub district by 52.75%, then Punung sub district by 14.29%, Kebonagung sub district by 9.89%, Pringkuku sub district by 6.59%, Pacitan sub district by 4.40%, and Sudimoro sub district, Arjosari sub district by 3.30% and Donorojo sub district by 2.20%. However, in Nawangan sub district, Bandar sub district, Ngadirojo sub district, there were no cases of Leptospirosis.

## C. Time

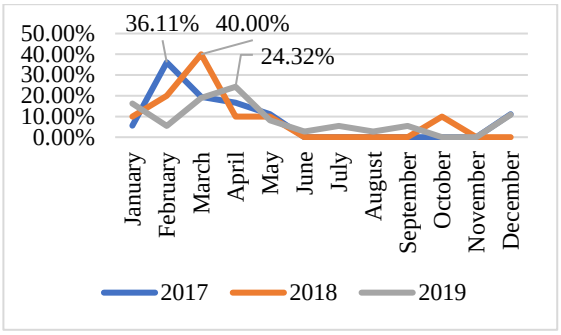


**Figure 7.** Distribution of Leptospirosis Cases by Time in Pacitan District in 2017–2019[8]

Based on Figure 7, the highest Leptospirosis cases in Pacitan district in 2017 occurred in February at 36.11%, in 2018 the highest Leptospirosis cases occurred in March at 40.0%, in 2019 the highest Leptospirosis cases occurred in April at 24.30%.

The rainy season in Pacitan district occurs in February–April and November–December, while the dry season in Pacitan district occurs in May–October.[20] In this study, it was stated that Leptospirosis cases that occurred in Pacitan district in 2017–2019 mostly occurred when rainy season occurs.

One of the risk factors for Leptospirosis is high rainfall. Heavy rainfall can cause waterlogging up to flooding. Leptospirosis can be transmitted through water contaminated with *Leptospira* bacteria.[24,25] Rains and floods are one of the factors causing Leptospirosis.[16,25,26]

The Maniiah et al[27] study showed that there was a relationship between the presence of standing water with the incidence of Leptospirosis and cases who are around the house there is standing water has a risk of 3,385 times greater exposed to Leptospirosis compared to respondents who are around the house there was no standing water.[27] Research conducted by Suwanpakde et al[28] in Thailand showeds a relationship between flooding and the incidence of Leptospirosis.[28]

## CONCLUSIONS

The most cases of Leptospirosis in Pacitan district in 2017–2019 occurred in male, the age group 40–49 years old, farmers and occurred in the rainy season, from February to April.

## ACKNOWLEDGEMENT

We would like to thank the Pacitan District Health Office for the secondary data on Leptospirosis reports.

## CONFLICT OF INTEREST

The authors state that they have no conflict of interest.

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***Review Article***

# The Curative Innovation of Novel Triple-Drug Compared to Double-Drug Regimen in Lymphatic Filariasis: A Systematic Review

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***ABSTRACT***

*The World Health Organization has established a global program for the elimination of lymphatic filariasis by 2020; recent data has shown an impracticable result accomplishing it. Therefore, this study aims to identify the efficacy and safety between triple-drugs (DEC, ALB, IVM) and double-drugs (DEC & ALB/IVM & ALB) for lymphatic filariasis treatment. A systematic review was conducted with Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement guidelines. The literature search was done using five databases: PubMed, ProQuest, ScienceDirect, EBSCO, and CENTRAL until December 3, 2020 without any publication date range imposed. Data collection was done by three independent reviewers and entered into a predesigned data extraction form. Cochrane risk of bias tool 2.0 was utilized in the quality assessment of the studies. Search strategies identified 209 studies. Three relevant full-text articles met our inclusion criteria. Overall studies had low risk of bias. The main findings are as follows: (a) Administration of single dose of triple-drug regimen resulted in a total elimination of microfilaria 12 months after treatment whilst 91% participants given with double-drug remained microfilaremic (p=0.002); (b) In larger samples (n=182), triple drug cleared microfilaria in 96% of the participants and only 32% of the participants receiving double-drug regimen after 12 months observation; (c) Statistically, the triple-drug safety has a lower degree than the double-drug regimen (p=0.02). The triple-drug treatment has a better efficacy compared to the double-drug regimen in treating lymphatic filariasis. Furthermore, both regimens are proven safe with no serious adverse events elicited.*

***Keywords:*** *albendazole; diethylcarbamazin; ivermectin; lymphatic filariasis; systematic review*

***ABSTRAK***

*Organisasi Kesehatan Dunia (WHO) telah menetapkan program global untuk mengeliminasi filariasis limfatik pada tahun 2020; data terbaru menunjukkan ketidakberhasilan pencapaian target tersebut. Oleh karena itu penelitian ini bertujuan untuk mengidentifikasi efikasi dan keamanan antara terapi menggunakan tiga obat (DEC, ALB, IVM) dan terapi dua obat (DEC & ALB/IVM & ALB) untuk pengobatan filariasis limfatik. Telaah sistematis dilakukan dengan pedoman pernyataan Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA). Pencarian literatur dilakukan melalui lima database: PubMed, ProQuest, ScienceDirect, EBSCO, dan CENTRAL hingga 3 Desember 2020 tanpa adanya batas rentang waktu publikasi. Pengumpulan data dilakukan oleh tiga peninjau secara independen dan dimasukkan ke dalam formulir ekstraksi data yang telah dirancang sebelumnya. Cochrane risk of bias 2.0 digunakan dalam penilaian kualitas studi.Strategi pencarian mengidentifikasi 209 studi. Tiga artikel yang relevan memenuhi kriteria inklusi studi. Keseluruhan studi memiliki risiko bias yang rendah berdasarkan penilaian penulis. Temuan utama adalah sebagai berikut: (a) Pemberian dosis tunggal pada terapi tiga obat mengeliminasi seluruh mikrofilaria setelah 12 bulan pengobatan sedangkan 91% peserta yang diberi terapi dua obat masih mengalami mikrofilaremia (p=0,002); (b) Dalam sampel yang lebih besar (n=182), mikrofilaria tereliminasi pada 96% peserta yang menerima*

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*terapi tiga obat dan 32% peserta yang menerima terapi dua obat setelah 12 bulan observasi; (c) Secara statistik, terapi menggunakan tiga obat lebih aman digunakan dibandingkan dengan terapi dua obat (p=0,02). Terapi tiga obat memiliki efikasi yang lebih baik dibandingkan dengan terapi dua obat dalam menangani filariasis limfatik. Kedua terapi juga terbukti aman tanpa adanya efek samping berat yang ditimbulkan.*

***Kata kunci:*** *albendazol; dietilkarbamazin; filariasis limfatik; ivermektin; telaah sistematis*

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## INTRODUCTION

Lymphatic filariasis (LF) is a severe manifestation caused by a parasitic infection of worms belonging to the genus Wuchereria and Brugia, that is transmissible by means of a mosquito vector.[1] This infection does not kill its host but significantly reduces their quality of life.[2] Latest data reported from the World Health Organization (WHO), which is in the year 2000, recorded over 120 million people were infected and about 40 million disfigured.[3] In Indonesia alone, the ministry of health reported over 14,000 people suffered from chronic filariasis (elephantiasis) in the year 2014.[4] There is still an estimate of 893 million people worldwide remain at risk of getting LF.[3] Thus, this spectrum of disease is considered globally as one of the many neglected tropical diseases (NTD) requiring further interventions.[5]

The Global Programme to Eliminate Lymphatic Filariasis (GPELF) was initially established by the World Health Organization (WHO) in 2000 with the aim to achieve global elimination of LF by 2020.[6] The proposed strategy includes the interruption LF transmission using mass drug administration as well as Managing Morbidity and Preventing Disability (MMPD) by providing access to primary recommended care. Latest reported data in 2019 on the progress of this program concluded a total of 37.3% are still at risk worldwide, which discourages the previous statement of it being completed in the year 2020.[7] Nevertheless, it is best to focus on the treatment rather than the prevention in order to minimize the damage being done in the meantime.

Currently, there are several regimens to treat LF.[8] The well-known single-drug therapy is using Diethylcarbamazine (DEC) with a dosage of 6 mg/kg.[9] Other drugs, such as Ivermectin (IVM) or Albendazole (ALB), rose to amplify the efficacy of DEC when combined.[10] However, some research found flaws in this double-drug therapy, a detection of microfilaria at one year posttreatment. A systematic review on the combination of ALBwith DEC also yielded little or even no differences compared[11] to using single-drug. In 2015, a pilot randomized controlled trial (RCT) was done with the hypothesis that a triple-drugcombination of DEC, ALB, and IVM might manifest a better result and coverage compared to only using two drugs.[12] The downside is that in accordance with medical scientific theory, more drug consumption is equivalent to more adverse events (AEs). Through this hypothesis, the objective of our systematic review is to analyze the efficacy and safety between a triple-drug and double-drug regimen in LF curative management qualitatively.

## METHODS

A systematic review was conducted with Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement guidelines to identify the efficacy and safety between a triple-drug and double-drug lymphatic filariasis treatment.[13] Population, Intervention, Comparison, and Outcomes (PICO) questions were also used to formulate the inclusion criteria of this systematic review. The answer of those questions consecutively: Patients confirmed

suffering from LF with no prior antifilarial treatment; Intervention with a triple-drug regimen consisting of DEC, ALB, and IVM; Comparison with a double-drug regimen consisting of DEC and ALB or IVM and ALB; Outcomes measured are efficacy and safety of both regimens. Differences between regimen doses and duration will be analyzed qualitatively based on the outcomes. The other inclusion criteria for this study is the study design and full-text availability. The study design had to be a RCT with participants confirmed as LF patients, and all included studies must have full text.

The literature search was done using five databases: PubMed, ProQuest, ScienceDirect, EBSCO, and CENTRAL with "*lymphatic filariasis*," "*Ivermectin*," "*Albendazole*," and "*Diethylcarbamazine*" as the main keywords until December 3, 2020 without any publication date range imposed. No language restrictions were imposed. The complete keywords are listed in Table 1 in the appendix. The result of the search was then imported to EndNote X9, and the duplicates were removed. All authors participated through each phase of the review independently by screening the titles and abstracts, assessing the full text for eligibility criteria, then including the relevant studies. Data collection was done by three independent reviewers (RR, EA, and C) and entered into a predesigned data extraction form. Differences arising between the three reviewers regarding study eligibility were resolved by consensus.

**Table 1.** Search Keywords

| Database | Keywords | Articles |
|---|---|---|
| PubMed | (((((((((Elephantiasis, Filarial[MeSH Terms]) OR (Elephantiasis, Filarial[Title/Abstract])) OR (Lymphatic Filariasis[Title/Abstract]))OR (Lymphatic Filariases[Title/Abstract])) OR (Bancroftian Filariasis[Title/Abstract])) OR (Malayi Filariasis[Title/Abstract])) OR (Elephantiasis[Title/Abstract])) AND ((((Ivermectin[MeSH Terms]) OR (Ivermectin[Title/Abstract])) OR (Ivomec[Title/Abstract])) OR(IVM[Title/Abstract]))) AND ((((albendazole[MeSH Terms])) OR (albendazole[Title/Abstract])) OR (ALB[Title/Abstract]))) AND (((Diethylcarbamazine[MeSH Terms]) OR(Diethylcarbamazine[Title/Abstract])) OR ("Diethylcarbamazine Citrate"[Title/Abstract])) | 105 |
| ProQuest | (ab(Elephantiasis, Filarial OR Lymphatic Filariasis OR Lymphatic Filariases OR Bancroftian Filariasis OR Malayi Filariasis OR Elephantiasis) OR ti(Elephantiasis, Filarial OR Lymphatic Filariasis OR Lymphatic Filariases OR Bancroftian Filariasis OR Malayi Filariasis OR Elephantiasis)) AND (ab(Ivermectin OR Ivomec OR IVM) OR ti(Ivermectin OR Ivomec OR IVM)) AND (ab(albendazoleOR ALB) OR ti(albendazole OR ALB)) AND (ab(Diethylcarbamazine OR Diethylcarbamazine Citrate) OR ti(Diethylcarbamazine OR Diethylcarbamazine Citrate)) | 44 |
| Science Direct | (Elephantiasis, Filarial OR Lymphatic Filariasis OR Lymphatic Filariases OR Bancroftian Filariasis OR Elephantiasis) AND Ivermectin AND albendazole AND (Diethylcarbamazine OR Diethylcarbamazine Citrate) | **27** |
| EBSCO | (ab(Elephantiasis, Filarial OR Lymphatic Filariasis OR Lymphatic Filariases OR Bancroftian Filariasis OR Malayi Filariasis OR Elephantiasis) OR ti(Elephantiasis, Filarial OR Lymphatic Filariasis OR Lymphatic Filariases OR Bancroftian Filariasis OR Malayi Filariasis OR Elephantiasis)) AND ( ab(Ivermectin OR Ivomec OR IVM) OR ti(Ivermectin OR Ivomec OR IVM)) AND (ab(albendazoleOR ALB) OR ti(albendazole OR ALB)) AND (ab(Diethylcarbamazine OR Diethylcarbamazine Citrate) OR ti(Diethylcarbamazine OR Diethylcarbamazine Citrate)) | 98 |
| CENTRAL | Elephantiasis, Filarial[MeSH Terms] AND Ivermectin[MeSH Terms]AND albendazole[MeSH Terms] AND Diethylcarbamazine[MeSH Terms] | 13 |

Cochrane risk of bias tool 2.0 was utilized in the quality assessment of the studieswhich covers the following seven domains ofrisk. Included study quality will be classified as low, unclear, or high risk of bias.[14] Disagreements arising in the process of the evaluation were all resolved by discussion among the review team.

## RESULTS AND DISCUSSION

### Search Results

A literature search from electronic databases yielded 287 studies. After removing the duplicates, 209 remaining studies. Screening through the titles and abstracts, the authors excluded studies with eight other studies that met the inclusion criteria. The result showed that three studies matched the criteria for inclusion.[12,15,16] Search flowchart and selection method was summarized in Figure 1.

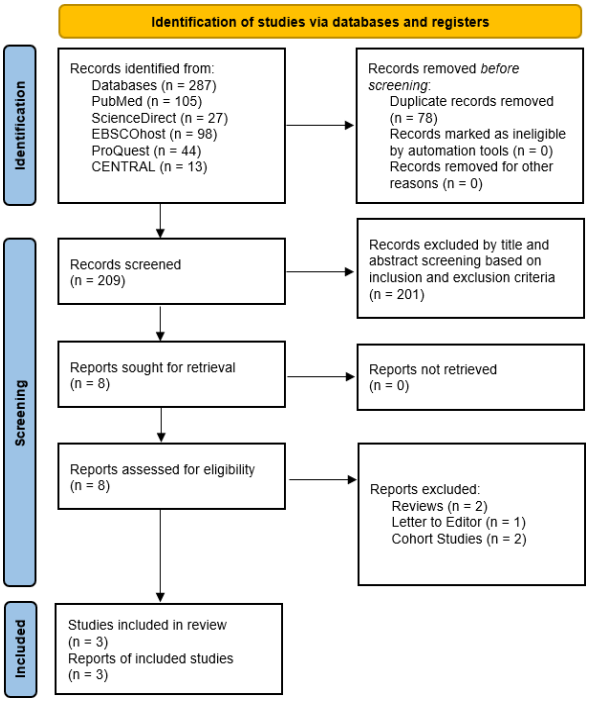


**Figure 1.** PRISMA Flow Diagram of the Identification and Selection of Studies Included in the Analysis

### Study Characteristics

All of the included studies were done using the RCT design. Inclusion and exclusion criteria across studies showed similarities. Most of the study populations included were from Papua New Guinea[12,15] and Côte d'Ivoire in West Africa[16] that showed small sample varieties. The regimen dosage and duration has slight differences amongst studies with efficacy and safety analysis. Detailed characteristics of the included studies were summarized in Table 2.

### Quality Assessment

There were unclear risks for all studies from the domain of selection and reporting bias. Concealment of the randomization done between the control and interventional group was unexplained.[12,15,16] Also, two studies excluded incomplete data from the statistical testing; thus can alter the results.[12,15] Two studies have an unclear risk in detection bias resulting from inadequate data.[12,15] Overall studies had a low risk of bias based on the author's judgement, was summarized in Figure 2.

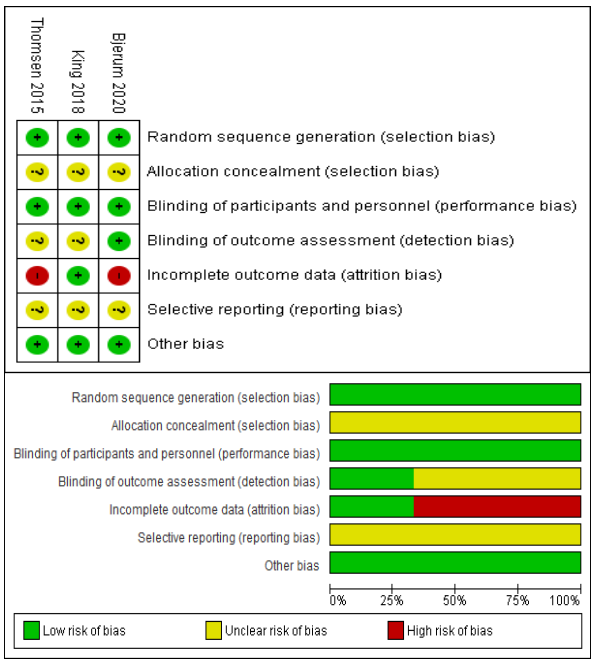


**Figure 2.** Risk of Bias Summary: Review Authors' Judgements about Each Risk of Bias Item for Each Included Study

### Study Characteristics

The pilot study done by Thomsen et al.[12] Included 24 participants with no chronic illnesses or prior LF infection. Participants

also have not got the MDA prophylaxis. Included participants divided into two parallel groups with every 12 participants that received the triple-drug (DEC + ALB + IVM) or the double-drug regimen (DEC + ALB). The study yielded higher efficacy in the triple-drug regimen with total eradication until 12 months follow-up than the double-drug regimen still resulting in 10 of 11 participants having positive microfilaremia (p=0.002). After two years of follow-up, both regimens showed complete eradication of microfilaremia.[12]

In 2018, another comparison of a RCT study done by King et al. is conducted to evaluate the efficacy of a single dose of triple-drug regimen in sustaining clearance being higher than a single dose of double-drug regimen for LF. A total of 182 participants were included, 60 of which were assigned to receive the triple-drug regimen administered once, and 61 for each double-drug regimen administered once and once a year for three years. Among 182 participants, 172 (95%) were evaluated at 12 months, 165 (91%) at 24 months, and 158 (87%) at 36 months after trial initiation–several were excluded for similar reasons, including a withdrawal, moved from the area, died, took a second dose by mistake or merely lost to follow-up. However, despite the decrease of the number of participants, the outcome of triple-drug regimen still result in significantly greater microfilarial clearance at 36 months than a single dose of the double- drug regimen, with a p-value lower than 0.025 (p=0.02), and was non-inferior to that with the double-drug regimen administered once a year for three years, with a one-sided P value for noninferiority lower than 0.025 (P=0.004).[15]

The most recent study was done in 2020 by Bjerum et al. consisting of 97 participants and using a similar triple-drug regimen but a somewhat different double-drug regimen consisting of IVM and ALB. The triple-drug group (45 participants) was given a single dose at the beginning of the research, while the double-drug group (52 participants) was given annually for three years. Results measured were the clearance of microfilaremia at 6, 12, 24, and 36 months posttreatment. The triple-drug regimen was significantly better compared to the double-drug regimen in clearing microfilaremia at 6 and 12 months (both with the value of p<0.001). However, superiority is the exact opposite in the 36 months (p=0.045). At the 24 months posttreatment, the triple-drug regimen was still better but with an insignificant value (p=0.53). This concludes that although the triple-drug group was only given once at the beginning of the study, it can maintain a better clearance up to 24 months posttreatment.[16]

**Table 2.** Characteristics of the Included Studies

| Author | Study Design | Samples | Interventions | | Duration of Follow-up | Result | |
|---|---|---|---|---|---|---|---|
| | | | Triple Drug | Double Drug | | Effectivity | Adverse Event |
| King et al., 2018 | Randomized Controlled Trial | N=182<br>This includes a total of 60 patients assigned IVM+DEC+ALB administered once, 61 to DEC+ALB administered once, and 61 to DEC+ALB once a year for 3 years | DEC + ALB + IVM<br>IVM 200 µg/kg plus DEC 6mg/kg plus ALB 400mg administered once at trial initiation | DEC + ALB<br>DEC 6 mg/kg plus ALB 400mg administered once at trial initiation | Follow-up was done at 12,24 and 36 months after trial initiation | Triple-drug regimen cleared microfilaremia in 55 (96%), 52 (96%) and 55 participants (96%) at 12, 24 and 36 months respectively.<br>A single dose of double-drug regimen cleared microfilaremia in 18 (32%), 31 (56%), 43 (83%) participants at 12, 24 and 36 months respectively. | Moderate AEs after the initial treatment were more frequent with the triple-drug regimen than with the double-drug regimen. |
| Thomsen et al. 2015 | Randomized Controlled Trial - Single blinded parallel - group | N=24<br>This includes a total of 12 patients assigned to DEC + ALB group and 12 patients assigned to DEC + ALB + IVM groups | DEC + ALB + IVM<br>Single dose IVM 200 µg/kg; ALB 400 mg; DEC 6 mg/kg | DEC + ALB<br>Single dose DEC 6 mg/kg; ALB | Follow up was done until 2 years posttreatment | Triple-drug regimen resulting total elimination of microfilaria after 36 h and 7 days treatments and remain so at 12 months posttreatment, than the double-drug regimen 10 of 11 participants remained microfilaremic at 12 months follow-up | No serious AE was found in both treatment groups. Most common AEs elicited were fever, myalgia, pruritus, and proteinuria/hematuria.<br>Adverse events were more on the triple-drug groups compared to the double- drug groups (83% and 50%, respectively; p=0.02). |
| Bjerum et al. 2020 | Randomized Controlled Trial | N=97<br>This includes a total of 52 patients assigned to IVM+ALB group and 45 patients assigned to IVM+DEC+ALB group | DEC + ALB + IVM<br>IVM 200 µg/kg and ALB 400 mg plus DEC 6 mg/kg were given only once at the beginning of the study. | IVM + ALB<br>IVM 200 µg/ kg and ALB 400 mg were given in a 3 annual dose. | Follow-up was done at 6, 12, 24, and 36 months posttreatment | Triple-drug regimen resulted in a Mf complete clearance of 89%, 71%, 61%, and 55% at a follow-up time of 6, 12, 24, and 36 months, respectively. While double-drug regimen resulted in a Mf complete clearance of 34%, 26%, 54%, and 79% at similar follow-up time, respectively. | Both groups resulted in similar AEs, which are none in the severe (grade 3) category and a similar one at the mild (grade 1) category. However, triple-drug therapy elicited more moderate (grade 2) AEs compared to double-drug therapy. |

**Abbreviation:**
AE, Adverse Events; ALB, Albendazole; ALT, Alanine Transferase; AST, Aspartate Transferase; DEC, Diethylcarbamazine; dl, Deciliter; g, Gram; IVM, Ivermectin; kg, Kilogram; mf, Microfilaria; mg, Milligram; ml. Milliliter; µg, Microgram; N, Number of Participants; N/A, Not Available

## Safety Regarding the Adverse Events

All three studies reported that triple- drug therapy resulted in a much more AE compared to double-drug therapy. This is supported by the basic medical lessons that more drugs are equal to greater side effects. However, the AEs reported are mild to moderate AEs like fever, myalgias, headache, nausea, and the like. There are no serious or severe (e.g. grade 3) AEs reported.[12,15,16]

This systematic review concludes a similar result found in all three studies.[12,15,16] All things considered, the triple-drug regimen was revealed for being the more effective option compared to the double-drug regimen in treating LF. The clearance of

microfilaremia measures this effectiveness at their given posttreatment, follow-up time. Study by Bjerum et al., however, showed that the double-drug regimen was significantly superior at 36 months posttreatment.[16] Another important aspect is the therapy's safety, which is measured by listing all AEs elicited after drug consumption. Both regimens are considered safe by means of no serious or severe AEs obtained. Mild to moderate AEs like fever, nausea, headache, and the like are more commonly found in patients treated with the triple-drug compared to the double-drug regimen.[12,15,16]

## Pharmacological Aspects between the Regimen

Each single drug has variability in their pharmacokinetics profile. Albendazole alone has poor absorption in gastrointestinal tract[17,19] compared with ivermectin or DEC that achieve peak plasma concentration faster than albendazole.[20] Well distribution is confirmed by each drug, but ivermectin has high lipophilicity,[21,22] opposites with DEC.[23,24] Metabolism of each drug occurs mostly in liver. Only DEC shows partial metabolism that slows down the elimination process. Most of the drugs excreted through feces and bile, less in urine. Nevertheless, lipophilicity profile that ivermectin has and partial metabolism in DEC reflects higher half-time for both drugs.[21,25]

Mechanism of action each drug also differs one to the other. Active metabolites of albendazole (albendazole sulfoxide) causes degeneration of cytoplasmic microtubules helminths with the decrease of parasite's glycogen stores.[17,18] Then, DEC yields sensitization of macrophage to microfilaria that ease the clearance process.[23,25] Lastly, ivermectin can causes hyperpolarization, however other study shows that ivermectin has the ability to sterilize the adult filarial.[26,27]

Combining of these drugs creates better pharmacokinetics and efficacy profiles because of uniqueness of each drugs. Using triple regimen shows superiority rather than double regimen. Half-life ($t_{1/2}$) and concentration max ($C_{max}$) higher if we add ivermectin in standard double regimen (DEC and ALB). Figure 3 illustrates slightly higher serum concentration of DEC in initial times after consumed if combined as triple-drug regimen. Similar profile also obtains from serum concentration of albendazole that increase in triple-drug regimen. Those pharmacological profiles raise the efficacy of triple-drug regimen than double-drug regimen.[12]

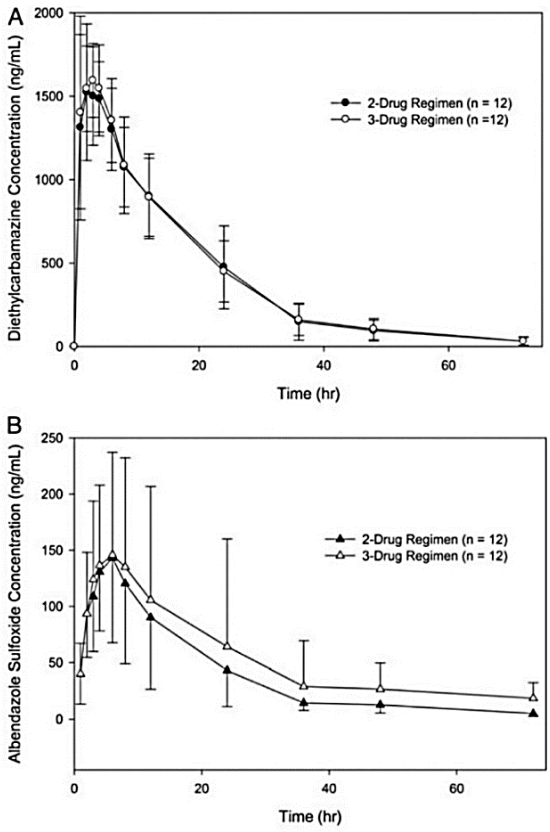


**Figure 3.** Concentration of lymphatic filariasis regimen in correlation with duration. (A) Mean serum plasma of DEC (B) Active metabolite of ALB[12]

Multimodal mechanism of actions also improves the efficacy of triple-drug regimen. Different sites of parasites killing process increase the chances of microfilaria deaths. Both albendazole and DEC can directly kill microfilaria. Addition to that, ivermectin will improves reduction of microfilaria with sterilization of adult worms that reflects lower microfilaremia level in longer times.

Nevertheless, further research is needed to carry out in determining definitive reasons whether it is due to additive or synergistic effect between the triple-drug interactions.[26]

Until now, each drugs using standard dose, even it already combined as triple-drug regimen. The fact that limitation arises from single drug with standard doses can be covered by other drugs. Adverse effects with all standard doses also depict low or minimal events. Nonetheless, specific research about dose adjustment still be needed. The high efficacy of triple-drug regimen can accelerate eradication of LF. This data is supported with a mathematical model that predicts for faster approaches to eliminate LF.[28]

**Difference in Various Aspects of the Regimen**

Although there are many similarities in the three included RCTs, their differences are a requirement to be discussed. Differences arise across studies yielded hazy analyzes in the overall results. The main distinguishable factor is the inconsistency in the drug combinations used. Bjerum et al. use a different double-drug combination, which includes ALB and IVM, compared with two other studies that used DEC and ALB.[16] A clinical trial in Ghana using a combination of ALB and IVM showed the same efficacy and safety with a controlled group (DEC and ALB) but statistically incomparable.[29] Moreover, a model-analysis results in the same efficacy between both kinds of double-drug regimen (IVM and ALB/DEC and ALB).30 Our analysis also considers IVM mechanisms of action that can be the main reason for DEC substitution in LF. As mentioned previously, ivermectin causes paralysis and death of parasites by interacting with its chloride channel in the cell membrane leading to hyperpolarization.[31,32] Although there are differences among the double-drug regimens, it can be concluded that both showed the same efficacy and safety that can be compared with triple-drug based on the data analyses and each authors' judgments.

Another difference comes from the regimen frequency in which the double-drug regimen is not only given once. This difference can be ignored since a higher frequency of the double-drug regimen still shows inferior outcomes compared to the triple-drug regimen.[23] Moreover, a study showed that the double-drug regimen has minor to none efficacious effect even with more frequency.[33] However, in some studies, it can affect the effectivity outcome on the last day of the treatment follow-up.16

In the safety objectives, a longer duration of follow up showed an extension of the safety observation. Although there are no serious AEs, the triple-drug regimen contains more chemical agents compound which can cause more AEs when compared to the double-drug regimen.[34,35] Risk vs benefit will be the primary consideration when it comes to LF treatment.[11] The highest benefits, besides the side effects, can be found by using the triple-drug regimen. However, in public situations, the doctor's explanations about the triple-drug regimen's potent benefits must be performed to prevent the misunderstanding of its AEs.

**Strength and Limitation of Each Study**

Included studies also shared some strengths and limitations. By referring to the risk of bias assessment, strengths such as the inclusion of using a random component had been performed, concluding a low risk of bias for random sequence generation, followed by the blinding of participants and the personnel or trial staff, specifically those who assessed the adverse events in the studies done by King et al. and Bjerum et al.[15,16] However, the blinding of outcome assessment was only low risk in the study done by Bjerum et al. since it was specified that although it was an open-label trial, the investigators and staff who evaluated the examinations were masked with respect to treatment arm assignments.[16] Meanwhile, the study done by King et al. gained the upper hand in incomplete outcome data, by conducting a sensitivity analysis (chi-squared analysis) to evaluate the potential effect of the missing data on the

primary outcome at 36 months.[15] There were also no other biases present.

Several inherent limitations are the questionable allocation concealment, as these three studies did not indicate the method to conceal the possibility of foreseeing the assignments. As mentioned before, blinding of outcome assessment is also unclear in the studies done by Thomsen et al. and King et al.[12,15] Another important caveat is the removal of participants which are lost to follow-up without performing a further analysis for the potential effects in the Thomsen et al. and Bjerum et al.'s study.[12,16]

King et al.'s study has also emphasized its limitations when assessing adverse events in the first 10 hours of follow-up since participants may have been aware of the treatment group assignments.[12] Although, the subsequent follow-up of the participants in their communities was performed in a blinded manner by different trial staff. Also, the detection of microfilaremia at follow-up could have been due to reinfection. On the other hand, the limitations from Bjerum et al.'s study include the unreliable outcome of higher infection rate in males due to its higher number of participants.[16] This study is also involved in the retreatment of Mf-positive individuals after 24 months following a single dose of triple-drug therapy; however, this number was later considered as a failure.

## SUMMARY

The objective of this systematic review leads to the conclusion that the triple-drug regimen is superior in terms of efficacy, but resulted in the sacrifice of its safety. One of the downsides in these three studies is the small geographic variety in the included respondents. It is crucial that future studies consider and involve a wide variety of people in order for this research to be generalized. To conclude, based on this review, the presence of a triple-drug regimen can really aid in improving the course of lymphatic filariasis; thus, leading to much better outcomes.

## ACKNOWLEDGEMENT

This endeavor would not have been possible without the generous support from our university. We would like to thank Atma Jaya Catholic University of Indonesia for the continuous encouragement, appreciation and keen interest in our research.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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***Original Article***

# A Prototype N95 Sterilizer: An Alternative Solution during Personal Protective Equipment Crisis

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***ABSTRACT***

*The high demand for N95 masks, especially during the COVID (Coronavirus disease)-19 pandemic, has caused shortages worldwide. This study aimed to examine the sterilization ability of the portable sterilizer prototype for N95 masks and its effect on the filtration ability and changes in air resistance on the N95 mask in order to thrift personal protective equipment (PPE) use during a shortage. The sample used was an N95 mask type 1860. The mask was contaminated with 0.6-0.8 MFU Staphylococcus aureus and Escherichia coli. The sterilization methods used were Ultraviolet Germicidal Irradiation (UVGI), Heat at 75°C, and a combination of both from 1 to 120 minutes. Next, the masks were cultured in a nutrient agar medium. For aerosol penetration and air resistance tests, masks were tested before and after the sterilization process, lasting from 5 to 60 minutes. This prototype sterilizer with Heat effectively killed E. coli and S. aureus starting from 3 minutes. The filtration ability of the N95 mask was maintained at >95% even after the sterilization process with 75°C heat, UVC, or a combination of both for up to 60 minutes. There was also no significant difference in air resistance between new masks and masks that had been sterilized using a portable sterilizer. This prototype sterilizer with Heat at 75°C can effectively sterilize against both gram-positive and negative bacteria in the N95 mask without reducing the aerosol filtration ability and changing the air resistance of the N95 mask.*

***Keywords:*** *aerosol; filtration; N95; Personal Protective Equipment; sterilization*

***ABSTRAK***

*Tingginya permintaan masker N95 terutama di masa pandemi COVID (Coronavirus disease)-19 menyebabkan kelangkaan masker di seluruh dunia. Penelitian ini bertujuan untuk menguji kemampuan sterilisasi dari prototipe portable sterilizer masker N95 dan pengaruhnya terhadap kemampuan filtrasi dan perubahan hambatan udara pada masker N95 dalam rangka penghematan penggunaan alat pelindung diri (APD) pada saat terjadi kelangkaan. Sampel yang digunakan adalah masker N95 tipe 1860. Masker dikontaminasikan dengan 0,6-0,8 MFU (McFarland unit) Staphylococcus aureus dan Escherichia coli. Metode sterilisasi yang digunakan adalah Ultraviolet Germicidal Irradiation (UVGI), panas pada suhu 75°C, dan kombinasi keduanya dalam durasi 1 hingga 120 menit. Selanjutnya, masker dikultur dalam media nutrien agar. Untuk uji penetrasi aerosol dan hambatan udara, masker akan diuji sebelum dan sesudah proses sterilisasi dengan durasi 5 hingga 60 menit. Prototipe sterilizer dengan panas 75 °C ini efektif membunuh E. coli dan S. aureus mulai dari 3 menit waktu sterilisasi. Kemampuan filtrasi*

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*masker N95 tetap terjaga >95% meskipun telah melalui proses sterilisasi dengan panas 75°C, UVC, atau kombinasi keduanya hingga 60 menit. Selain itu, tidak ada perbedaan yang signifikan dalam hambatan udara antara masker baru dan masker yang telah disterilkan menggunakan alat sterilisasi portabel. Prototipe alat sterilisasi dengan panas pada suhu 75°C ini dapat secara efektif mensterilkan bakteri gram positif dan negatif pada masker N95 tanpa mengurangi kemampuan filtrasi aerosol dan mengubah hambatan udara masker N95.*

***Kata kunci:*** *aerosol; Alat Pelindung Diri; filtrasi; N95; sterilisasi*

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## INTRODUCTION

Infectious diseases are one of the leading causes of death in the world. The World Health Organization (WHO) reports that lower respiratory tract infections are the fourth leading cause of death globally and the second most common cause of death in developing countries.[1] The easy transmission of disease from animals to humans or fellow humans makes infectious diseases have a reasonably high incidence. One method of transmission that transmits very quickly is through aerosol or airborne. This transmission occurs when an infected person expels droplets or aerosols when talking, singing, coughing, or sneezing.[2,3] One way to prevent this disease's transmission is using face masks. The use of face masks can reduce a person's chance of being infected by up to 90%.[4] One type of face mask is recommended for health workers as personal protective equipment (PPE) on duty is the N95 mask. The high demand for N95 masks, especially during the COVID-19 pandemic, has caused shortages worldwide.[5]

The Center for Disease Control and Prevention (CDC) publishes guidelines for reusing N95 masks during the PPE crisis. This reuse must pay attention to several things regarding N95 masks. Some things that need to be considered in mask reuse are contamination and filtration performance. In addition, it is also necessary to pay attention to the mask damage and its fitting.[6] Many studies have been conducted on the sterilization methods of N95 masks for reuse. Some methods studied were evaporation, dry heat, Ultraviolet C (UVC), gamma radiation, hydrogen peroxide, boiling in water, and liquid disinfectants such as chlorine and alcohol.[7–9] Some of these methods damaged the mask, but UVC and dry heat were reported to maintain a safe mask's filtration performance.[7]

Based on this problem, we designed a special portable sterilizer for N95 masks, which can eliminate pathogens but does not affect mask performance. This sterilizer is compact, easy to use, and can be used anywhere.

## MATERIALS AND METHODS

### Materials & Tools

The masks used were the N95 type 1860 3M masks. *E. coli* ATCC 25922 and *S. aureus* ATCC 6538 isolates were used to contaminate the masks. Particle counter HTI type HT9600, Manometer HT-1890, and Nebulizer OMICRON MY-520A were used as aerosol generators. The particle counter could measure aerosol particles starting from 0.3 µm, 2.5 µm, to 10 µm. This device also had a maximum measurement capability of $10^7$ piece/L particles and resolution up to 1 piece/L.

### Methods

#### Prototype Design of Portable Sterilizer

This portable sterilizer was made using a 15 mm Medium Density Fiberboard (MDF). Dimensions were about 37 cm high, 21.8 cm wide, and 21 cm depth. Inside the sterilization chamber, a wire mesh was placed as a base, and hangers were attached on the sides so that the masks could be hung vertically on the side

walls so that all masks could get an even heat between one another. The prototype of the portable sterilizer is shown in Figures 1A and 1B.

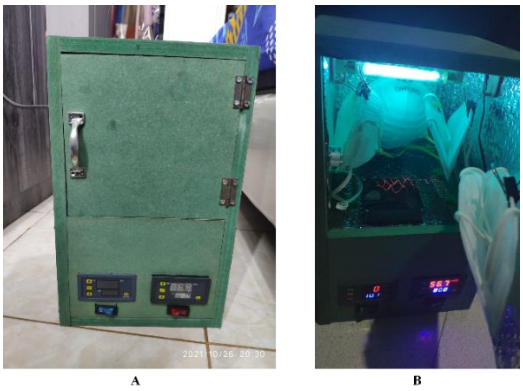

**Figure 1.** Portable sterilizer (A) Front view, (B) Inside view of portable sterilizer prototype when operating

This device used the heat method for sterilization resulting from converting electrical energy into heat. The heating element used was Nichrome Ni80 wire (80% nickel, 20% chromium). A 4W UVC lamp was also added to maximize the sterilization effectiveness.

The power source of this tool used a 240 W power supply with a 12 V DC and 20 A current. This tool could produce 200 W of thermal energy to heat the sterilization chamber by radiation. A 9 cm fan would help circulate the hot air produced by the heating element, so the heat was more evenly distributed throughout the sterilizer chamber. The thermostat was used to control the heat. It was set on at 75°C and off at 75.5°C. A timer was used to adjust the duration of sterilization according to the treatment group.

### Mask Filtration Efficiency Tester Design

The mask filtration efficiency tester was designed as shown in Figures 2A and 2B. This tool was tried to imitate the working principle of the standard tool for measuring mask filtration capability, TSI Automated Filter Tester 8130A. This tool was made from polyvinyl chloride (PVC) tube with an inside diameter of about 6.35 cm (2.5 Inches) and a length of about 65 cm.

The aerosol produced by the nebulizer would be mixed with room air using a blower which would then be blown into the intake of the filtration tester tool. The nebulizer will automatically generate a variable-sized particle. These particles' sizes will be distinguished using particle counter and calculated in numbers. The vacuum fan would suck the air that had been mixed with the NaCl aerosol. The mask would be placed in the middle of the tool to filter out aerosol particles that had been sucked. Particle counters were placed in space before filtration occurred or in front of the mask (Upstream) and space after the air was filtered or behind the mask (Downstream). In addition, Manometer sensors were also placed in the two chambers to measure the air pressure difference between chambers.

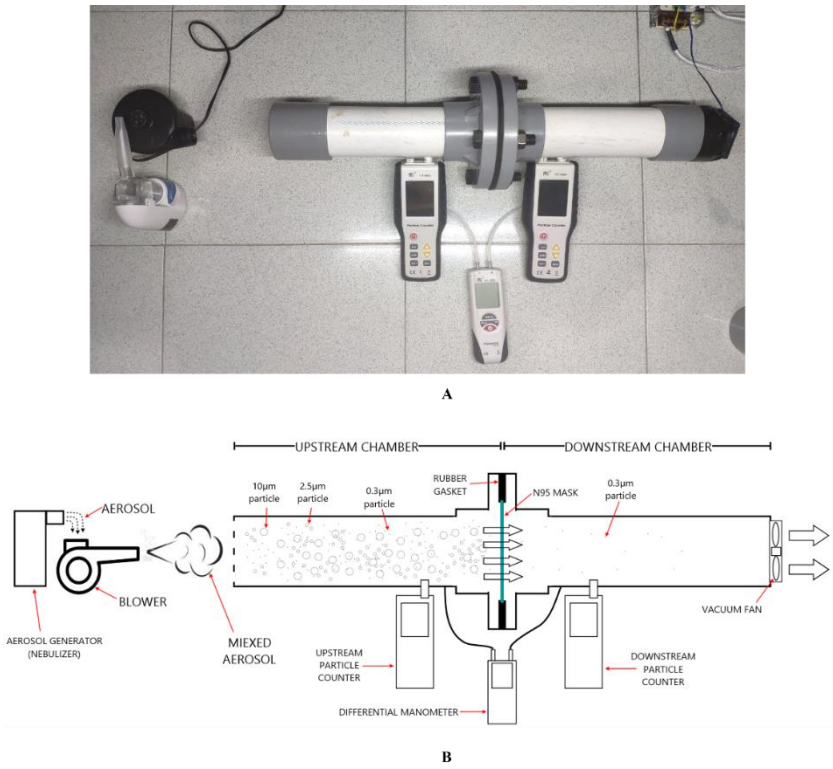


**Figure 2.** The mask filtration efficiency tester (A) Overall view, (B) The working principle of the mask filtration efficiency tester

## Sterilization Test

The experiment was conducted in Microbiology Laboratory, Hasanuddin University Hospital, Makassar, Indonesia. The masks were artificially contaminated by the method used by Ibáñez et al. [10] with modifications. Airborne pathogens could not be used in this study due to limited laboratory biosafety availability. In this investigation, *E. coli* and *S. aureus* microorganisms were employed instead. The mask was cut into small pieces about 20 x 7.5 mm so that it would later fit into the microcentrifuge tube during the elution process. All mask samples were clamped using a wooden clamp, put in a clear plastic bag, and pre-sterilized at 90°C for 60 minutes to eliminate environmental contamination. The mask was removed from the plastic and contaminated with 100 µl of a 0.6-0.8 MFU (McFarland Unit) solution of *S. aureus* or *E. coli*.

After that, the mask was put back into a plastic bag and the sterilization process was carried out using (1) UVC, (2) 75°C heat (temperature to inactivate *S. aureus* and *E. coli*),[11,12] and (3) a combination of both in a duration of 1, 3, 5, 10, 30, 60, 90, and up to 120 minutes. For the control, we used an uncontaminated mask as a negative control and an unsterilized mask as a positive control.

After the sterilization process was complete, the mask was drowned in 0.5 mL of saline solution in a microcentrifuge tube and vortexed to elute the bacteria contained in the mask. The saline solution was then dropped as much as 0.1 mL onto a nutrient agar medium and spread. The medium was incubated at 37°C for 24 hours. After 24 hours, bacterial growth would be observed. Culture results showing more than 30 colonies were categorized as positive, while less than 30 were categorized as negative because they were too few to represent the sample. Cultures that produced more than 300 bacterial colonies were considered too many to count (TMTC).[13]

**Aerosol Penetration and Air Resistant Test**

In preparation, the stiff edges of the mask were cut to remove the rigid structure of the mask. This was intended to make it easier for the mask to be inserted into the filtration test device later. After that, the mask was tested for its filtration ability (F) by calculating the difference in the number of 0.3 µm sized particles between the Upstream (Us) and Downstream (Ds) spaces using the formula:

$$F = \frac{(Us - Ds)}{Us} x100$$

Air resistance was tested by calculating the difference in air pressure in the Us and Ds chambers. After obtaining the initial data, the mask was sterilized using UVC, 75°C heat, or a combination of both for 5, 10, 30, and 60 minutes, respectively. After the sterilization process, the mask was tested again for its sterilization ability and air resistance as in the previous method. The results were about 20 x 7.5 mm compared before and after the sterilization process. The air pressure difference data were entered into Microsoft Excel software and tested using a paired T-test to find their significance. This method was a modification of the method used by Gobi et al.[14] and Vossen et al.[15] to determine the mask's filtration efficiency and air permeability. Briefly, the research flow is depicted in Figure 3.

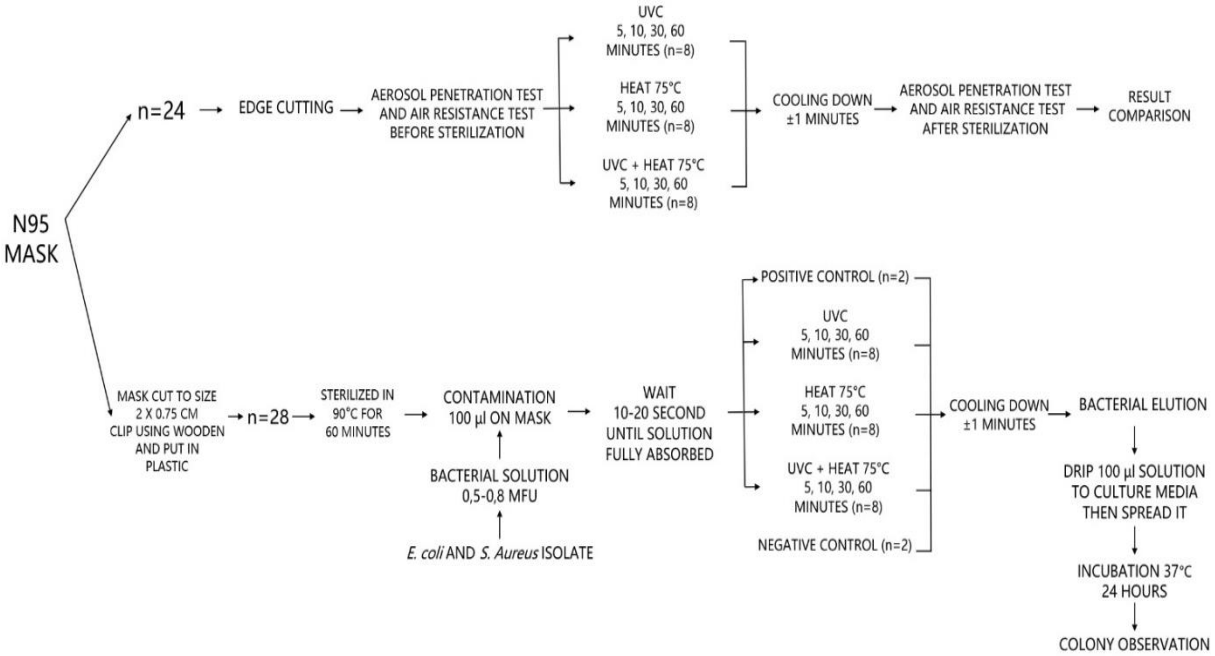


**Figure 3**. Research Flow

## RESULTS AND DISCUSSION

### Sterilization Test

Most of the culture results made more than 300 colonies of bacteria and were considered Too Many To Count (TMTC). More than 30 colonies were categorized as positive results, while less than 30 were categorized as negative because they were too few to represent the sample. Some plates also showed the results of colonies stacking up on each other due to the uneven distribution of eluted solution during preparation.

The results of mask culture after sterilization using the N95 prototype sterilizer are summarized in Table I. Using a portable sterilizer with the heat of 75°C gave negative culture results for both gram-positive and gram-negative bacteria from 5 minutes to 60 minutes of sterilization duration, as shown in Figure 4.

**Table 1.** Mask Culture Results after Sterilization using the N95 Prototype Sterilizer

| Sterilization Method | Culture Result | |
|---|---|---|
| | ***E. coli* (0.81 MFU)** | ***S. aureus* (0.7 MFU)** |
| Positive Control | **Positive** | **Positive** |
| Negative Control | Negative | Negative |
| UVC 5 minutes | **Positive** | **Positive** |
| UVC 10 minutes | **Positive** | **Positive** |
| UVC 30 minutes | **Positive** | **Positive** |
| UVC 60 minutes | **Positive** | **Positive** |
| Heat 75°C 5 minutes | Negative | Negative |
| Heat 75°C 10 minutes | Negative | Negative |
| Heat 75°C 30 minutes | Negative | Negative |
| Heat 75°C 60 minutes | Negative | Negative |
| UVC + Heat 75°C 5 minutes | Negative | Negative |
| UVC + Heat 75°C 10 minutes | Negative | Negative |
| UVC + Heat 75°C 30 minutes | Negative | Negative |
| UVC + Heat 75°C 60 minutes | Negative | Negative |

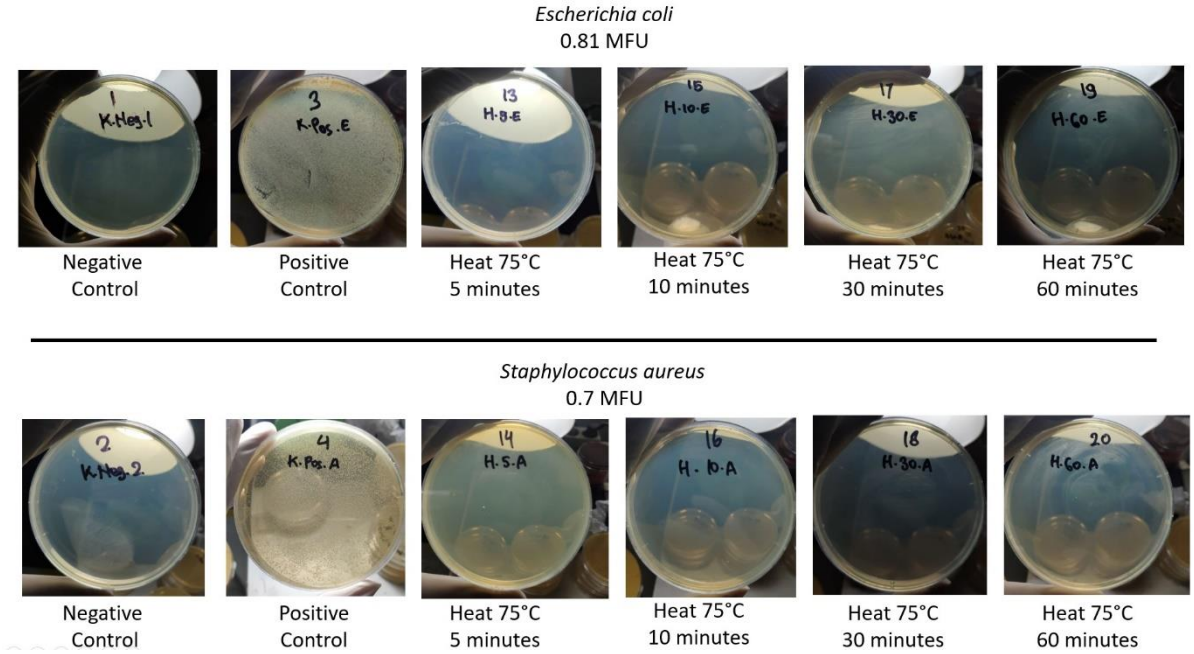


**Figure 4.** Mask Culture Results after Sterilization using heat of 75°C

We tried to reduce the duration of heat exposure to 1 and 3 minutes, respectively, to see the lower limit of this portable sterilizer's performance. At 1 minute, there was still colony growth, especially in *E. coli* culture, while in the 3 minutes group, the two groups of bacteria did not show any colony growth on the medium.

In contrast, sterilization using UVC gave the opposite result. This portable sterilizer could not eradicate *S. aureus* and *E. coli* even with 60 minutes of sterilization. The documentation of the mask culture results after sterilization using the prototype N95 with the UVC method is shown in Figure 5.

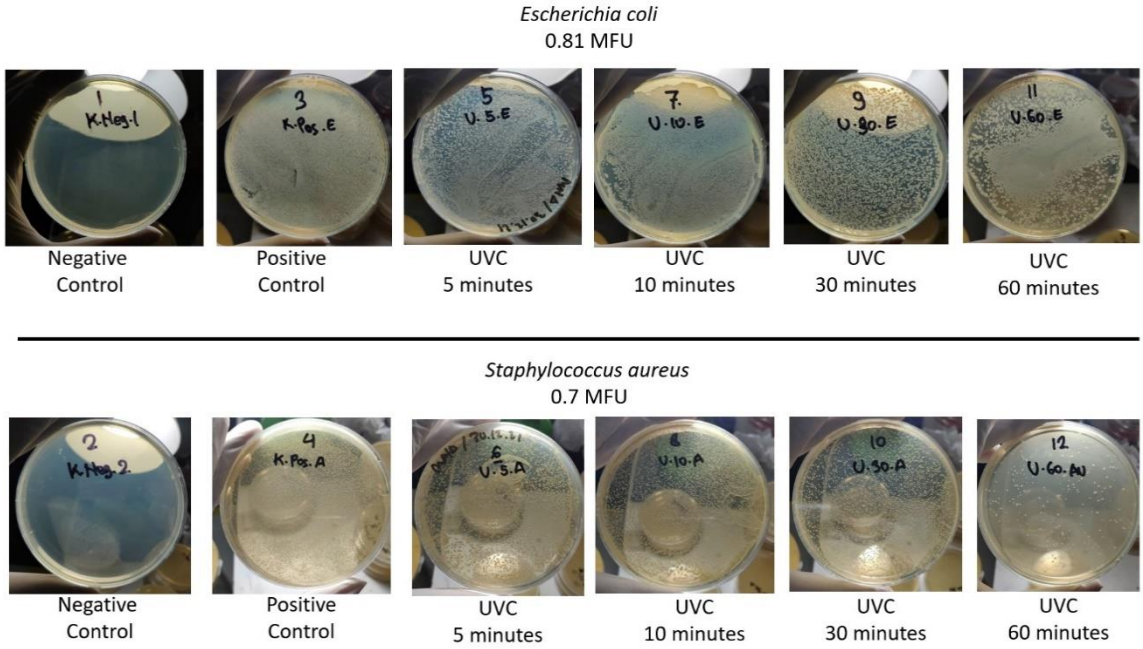


**Figure 5.** Mask Culture Results after Sterilization using UVC

Therefore, we added the duration of the mask's exposure to UVC rays to 90 to 120 minutes, but that still was not able to give negative culture from both bacteria, as shown in Table 2. Culture result documentation can be shown in supplementary Figure 6.

Using a combination of heat at 75°C and UVC gave no different culture results than using heat alone. Culture documentation is shown in Figure 7. Culture testing was done in a duplex to get more accurate results. There was no difference in culture results between the first and second experiments.

**Table 2.** Mask Culture Results in Shortened and Extended Sterilization Durations

| Sterilization Method | Culture Result | |
|---|---|---|
| | ***E. coli* (0.64 MFU)** | ***S. aureus* (0.82 MFU)** |
| Positive Control | **Positive** | **Positive** |
| Negative Control | Negative | Negative |
| Heat 75°C for 1 minute | **Positive** | Negative[1] |
| Heat 75°C for 3 minutes | Negative | Negative |
| UVC + Heat 75°C 1 minute | **Positive** | Negative |
| UVC + Heat 75°C for 3 minutes | Negative | Negative |
| UVC 90 Minutes | **Positive** | **Positive** |
| UVC 120 Minutes | **Positive** | **Positive** |

[1]only a colony was found

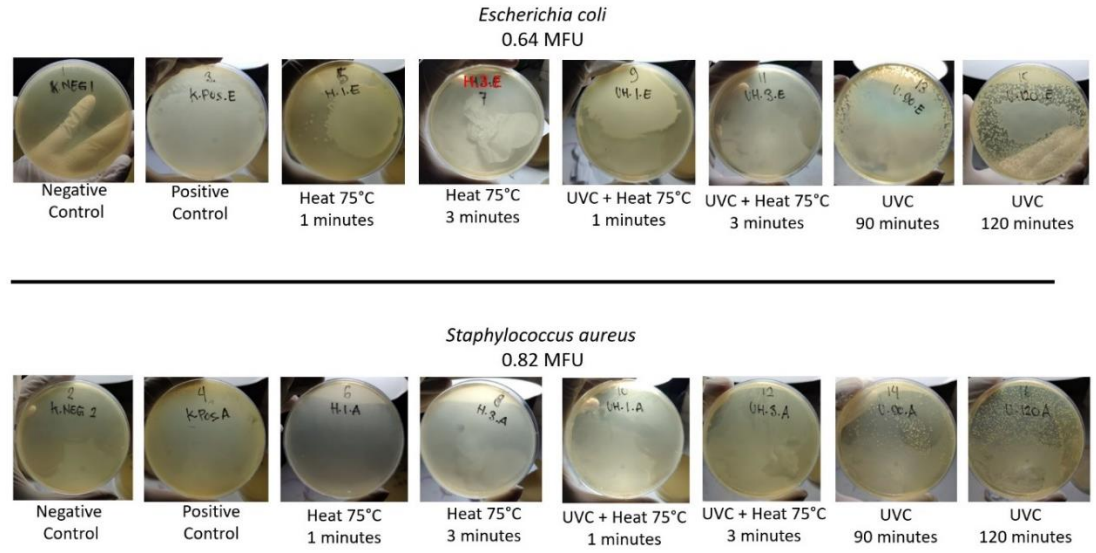


**Figure 6**. Mask culture results in shortened and extended sterilization durations

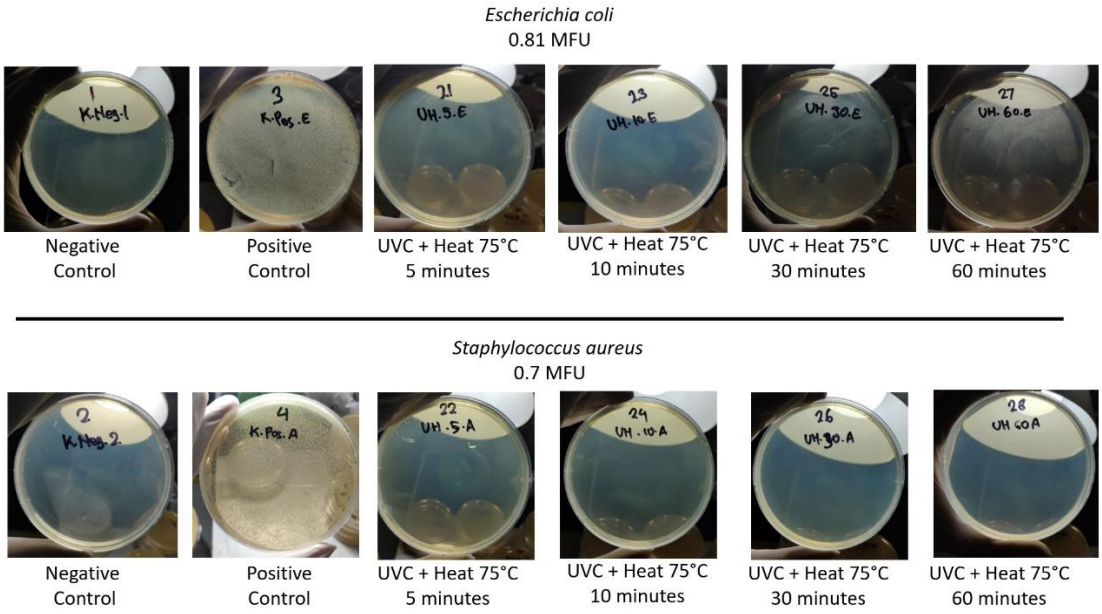


**Figure 7.** Mask Culture Results after Sterilization using both UVC and Heat

**Aerosol Penetration and Air Resistance Test**

The filtration ability of the N95 mask was maintained at >95% even though it had been through a sterilization process with 75°C heat, UVC, or a combination of both for up to 60 minutes Figure 8. In terms of air resistance, there was also no significant difference ($\rho$=0.07–0.50) between new masks and masks that had been sterilized using a portable sterilizer as shown in supplementary Figure 9.

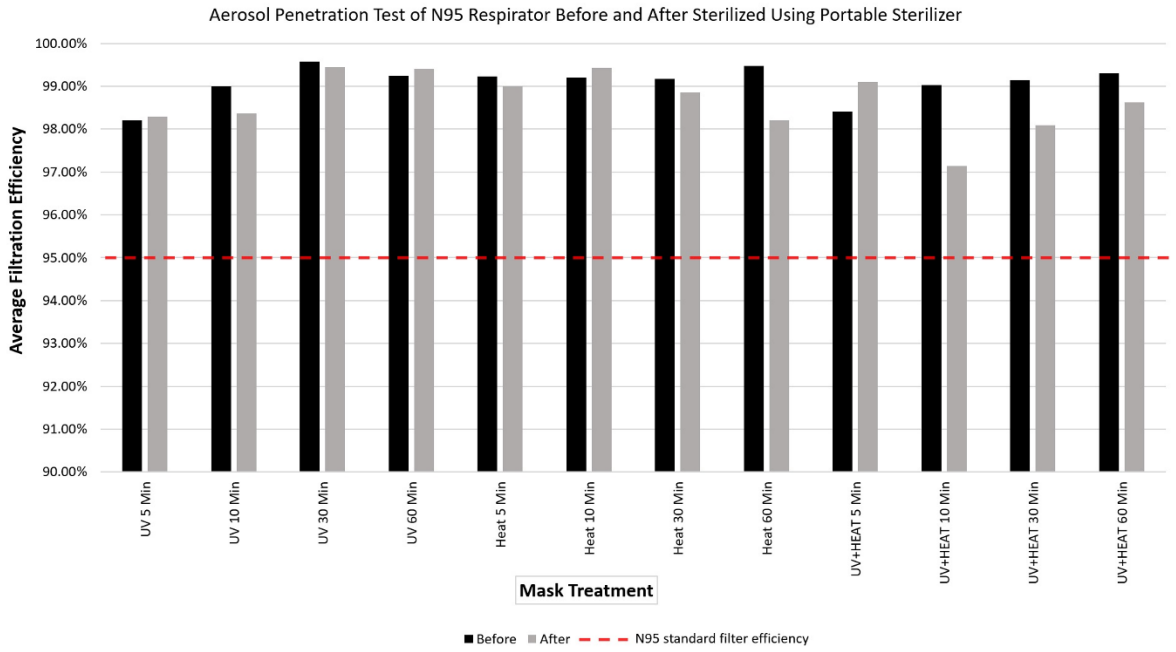


**Figure 8.** Comparison of N95 masks aerosol filtration efficiency before and after sterilization using the portable sterilizer

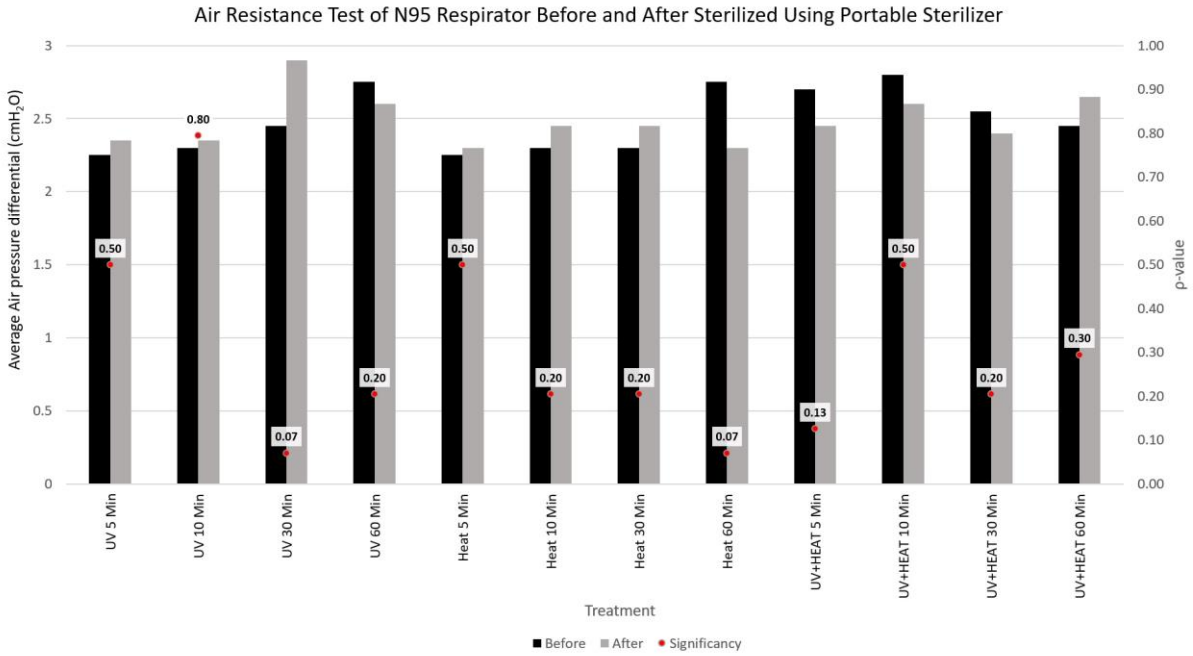


**Figure 9**. Comparison of Air Resistance of N95 Masks before and after Sterilization using the Portable Sterilizer

Based on the sterilization ability test results, using a portable sterilizer with the heat method was more effective in eradicating *S. aureus* and *E. coli*. This method killed the bacteria on the mask pieces starting by heating for 3 minutes, while UVC still gave positive culture results even though the mask pieces had been exposed to UV light for 2 hours. This result shows that the use of heat in the portable sterilizer is more effective when compared to the use of UVC rays. The combination of heat and UVC also gave a

negative result on the culture results. Thus there was no need to use both methods because it was just a waste of energy.

One of the factors that increase heat sterilization capability is humidity. The use of moist heat is more effective than the use of dry heat.[16] Humidity in tropical countries like Indonesia is relatively high. In this experiment, the humidity level in the room was around 55–60% RH. This high humidity environment increased the effectiveness of the heat sterilization capability of this portable sterilizer without the need for modification of the humidity level in the sterilization chamber. Other studies have also shown that heat is more effective than treatment using UVC for the sterilization of N95 masks.[17]

Bacteria's walls composed of protein have thermophobia characteristics. High temperatures will cause denaturation of these proteins and result in the death of these microorganisms.[18] *E. coli* at 60°C will die within 2.9 minutes.[19] Meanwhile, *S. aureus* will die at a temperature of 60°C for about 4.8–6.6 minutes.[20] For comparison, heating containing *SARS CoV-2* media at 65°C for 3 minutes is recommended to kill the COVID-19 virus.[21] However, *Mycobacterium tuberculosis* needs a higher temperature at 80°C to lose its viability.[22] But, it should also be considered that too high temperatures can damage the structure of N95. The polypropylene layer on the N95 mask has a melting point of 130–171°C.[23] If the temperature is too close, the structure will be damaged, impacting its filtration performance. Heating at a temperature of 125°C can reduce the filtration ability of N95 up to 90%.[7] We had tried to use an autoclave for the initial sterilization process to remove the environmental contamination of the mask sample before it was artificially contaminated. However, the mask sample showed a physical deformity like melting after being removed from the autoclave. Although it has excellent germicide capabilities, using an autoclave was not recommended in sterilizing N95 masks.[24]

The filtration ability of N95 is obtained by utilizing a combination of polypropylene microfibre and electrostatic charges. The name N95 was given because this mask could filter at least 95% of solid and aerosol particles in laboratory trials. The letter N indicates that this mask cannot filter oil-based vapor.[25] The N95 mask consists of several layers, one of which is a layer made of nonwoven polypropylene fiber with a diameter of 4.2 ± 3.9 µm, forming a layer with a thickness of 200-400 µm.[26,27] The sterilization process must maintain the electrostatic charge of this membrane so that the mask filtration performance does not decrease.

Golovkine et al. showed a decrease of 3 log concentrations of *SARS CoV-2* on the N95 surface after sterilization using UVC light at 1 mW/cm$^2$ for 10 minutes.[28] This result could be achieved because the UVC light source they used was very close and right on the mask's surface and back, leading to an adequate UVC exposure. In contrast to this portable sterilizer, the lack of effectiveness UVC is thought to be the result of the device configuration. The mask was placed in a hanging position on the side of the sterilization chamber, while the UV light source only came from above, resulting in uneven exposure to UVC rays and a blind spot in the sterilization process. In addition, this study did not measure the UVC radiation exposure dose, so that the UVC dose may be too low. However, exposure of the mask to UVC rays at an excessive dose can also reduce the filtering performance of the mask; thus, a precise dose is required.[29]

This sterilizer was designed because the heat source was located directly at the bottom of the sterilization chamber. If the mask was placed directly under the UVC source, in the bottom position, it was feared that the mask would melt because it was so close to the heating source. Thus, the mask must be hung on the side. However, the advantages of this configuration make this tool capable of loading a total of four masks in one

sterilization process, making it more efficient in operation.

In terms of an aerosol penetration test, using a portable sterilizer did not reduce the mask's filtration performance below 95%, whether using UVC, heat, or a combination of both, even after being exposed for 1 hour. This study also showed no significant change in the air resistance of the N95 mask in all sterilization methods for 1 hour, so the user was still comfortable breathing when using a sterilized mask. Another study also provided a similar result. Xiang Y *et al.* reported that exposure to dry heat to N95 at a temperature of 60°C and 70°C for 1 hour killed seven strains of bacteria and fungi, including *E. coli* and *S. aureus,* without reducing their filtration ability below 95%.[30] Even the use of heat up to 100°C for 5 minutes repeated 20 times did not affect the filtration ability of the N95 mask. [7]

## CONCLUSIONS

It can be concluded that this portable sterilizer was able to kill *E. coli* and *S. aureu*s in the N95 mask using the 75 °C heat method for 3 minutes without negatively affecting the filtration performance and air resistance.

Although the effect was shown starting from 3 minutes, we recommend using this portable sterilizer with the heat method with a minimum duration of 5 minutes to compensate for the time that this tool takes to raise the temperature from room temperature (25 °C) to operational temperature (75°C). In addition, this mini sterilizer is only for emergencies, such as when there is a shortage of N95 masks. However, it is much safer to use a new mask than a mask that has undergone sterilization.

The advantage of this tool is that it is smaller, compact, portable, and easy to use compared to the tools used in previous studies, which mainly used tools that were generally used on a commercial scale. The design itself still needs much improvement. Form mask placement needs to redesign, so it is safe from heat sources. Besides, the operating system needs to be changed from analog to digital so that the timer set becomes easier to set, and the exterior design must be updated to make it look contemporary. In addition, many more tests are needed regarding the effect of using a mini sterilizer on N95 masks, such as the impact of repeated use on masks, its effect on microscopic N95 fibers, the elasticity of mask strap rubber and fitment test, calculation of UVC doses, and the effect on various airborne pathogens such as *M. tuberculosis* and *SARS CoV-2.*

## ACKNOWLEDGEMENT

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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***Original Article***

# Antibiotic Sensitivity Against *Klebsiella* spp. in the Post Debridement Culture an Open Fracture in Emergency Department of dr. Soebandi Hospital Jember

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***ABSTRACT***

*Surgical site infection (SSI) in open fracture is often caused by bacterial contamination in the management of open fracture. Because of that, one of the most important thing in handling open fracture is debridement. Prophylactic antibiotics given are Cephalosporin and Aminoglycosides. Post-debridement culture is important in predicting the incidence of infection. One of the bacteria that is often found in post-debridement culture is Klebsiella spp. which can produce ESBL to fight β-lactam class of antibiotics. The purpose of this study was to determine antibiotic sensitivity against Klebsiella spp. in the post-debridement culture of cases of open fractures in the emergency department of dr. Soebandi hospital Jember. This study uses a laboratory exploratory research design. The sample of this study was the isolate of Klebsiella spp. which amounts to 5 from post debridement culture of open fracture patients in the emergency department of dr. Soebandi hospital Jember from March to May 2019.The method used is diffusion (Kirby Baurer) by matching using the CLSI standard table to determine sensitive, intermediate, or resistant. The results of this study showed that most antibiotics had resistance to Klebsiella spp., including β-lactam antibiotics, such as Amoxicillin, Ceftriaxone, Cefixime, Penicilin, Meropenem, and Cefadroxil. Vancomycin antibiotics are still sensitive to Klebsiella spp. in all patients. Gentamicin, Ciprofloxacin, Tetracycline, and Chloramphenicol antibiotics were sensitive in 1 patient. Erythromycin intermediates antibiotics against Klebsiella spp.. The conclusion of this study is that all β-lactam group antibiotics are resistant to Klebsiella spp while the most sensitive antibiotic is Vancomycin.*

**Keywords:** *antibiotic sensitivity; ESBL; Klebsiella spp.; open fracture; post debridement*

***ABSTRAK***

*Infeksi luka operasi pada patah tulang terbuka seringkali disebabkan oleh kontaminasi bakteri pada manajemen patah tulang terbuka. Oleh karena itu, salah satu hal yang penting pada penanganan patah tulang terbuka adalah debridemen. Kultur setelah debridemen penting dalam memprediksi kejadian infeksi. Salah satu bakteri yang sering ditemukan pada kultur setelah debridemen adalah Klebsiella spp. yang dapat menghasilkan Extended-spectrum β-lactamase (ESBL) untuk melawan antibiotik golongan β-lactam. Tujuan penelitian ini untuk mengetahui sensitivitas antibiotik terhadap Klebsiella spp. pada kultur post debridement kasus patah tulang terbuka di Emergency department dr. Soebandi hospital Jember. Penelitian ini menggunakan desain penelitian eksploratif laboratorik. Sampel penelitian ini adalah isolat bakteri Klebsiella spp. hasil kultur post debridement 5 pasien patah tulang terbuka di*

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*Emergency department dr. Soebandi hospital Jember periode Maret-Mei 2019. Metode yang digunakan adalah difusi (Kirby Baurer) dengan hasil pengukuran yang dikonversikan dengan tabel standar Clinical Laboratory Standards Institue (CLSI) untuk menentukan sensitif, intermediet, atau resisten. Hasil: Hasil penelitian ini menunjukan sebagian besar antibiotik mengalami resistensi terhadap Klebsiella spp., termasuk antibiotik golongan β-lactam, seperti Amoxicillin, Ceftriaxone, Cefixime, Penicilin, Meropenem, dan Cefadroxil. Antibiotik Vancomycin masih sensitif terhadap Klebsiella spp. pada seluruh pasien. Antibiotik Gentamicin, Ciprofloxacin, Tetracycline, dan Chloramphenicol sensitif pada 1 pasien. Antibiotik Erythromycin intermediet terhadap Klebsiella spp. Kesimpulan dari penelitian ini adalah semua antibiotik golongan β-lactam resisten terhadap Klebsiella spp. sedangkan antibiotik yang paling sensitif adalah Vancomycin.*

***Kata kunci:*** *ESBL; Klebsiella spp.; patah tulang terbuka; sensitivitas antibiotik; setelah debrimen*

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## INTRODUCTION

Surgical site infection (SSI) in the open fracture is often caused by bacterial contamination in open fracture management. Because of that, debridement is one of the most important things in handling open fractures. An open fracture is a break in the continuity of the bone with injury to the skin above the site of fractures, with traffic accidents the most cause.[1–3] In open fractures, contact with the outside environment is susceptible to infection. In the treatment of open fractures, one of the important things to do is debridement.[4–7]

In a study at Third Hospital of Hebei Medical University, most SSIs (81.8 %,18/22) were found during subsequent hospitalizations. The total incidence of SSIs was 6.0 % (22/364). The superficial SSIs accounted for 2.4 % (9/364) and deep SSIs for 3.6 % (13/364).[8], whereas in The Second Hospital of Tangshan, the overall incidence of SSI was 18.6%, with 17.0% and 1.6% for superficial and deep infection, respectively. There were 2027 males and 665 females of the study sample.[9]

Post-debridement culture is more important in predicting infection incidence than pre-debridement culture. In post-debridement cultures of open fractures, infections are often caused by Gram-negative bacteria such as *Klebsiella* spp., *E. coli*, *Pseudomonas* spp., *Acinetobacter* spp., and *Enterobacter* spp..[10,11] Research by Sitati et al. (2017) mentioned that bacteria found in post-debridement culture of open fractures are *Klebsiella* spp., *S. aureus*, *Pseudomonas* spp., CON (negative coagulase) Staphylococci, and *E. coli*. This is in line with the preliminary study conducted from March to May 2019 at the Emergency department dr. Soebandi hospital Jember, in 30 patients with open fractures, it was found that in the culture of post-debridement, the most common bacteria were *Klebsiella* spp.[12]

*Klebsiella* spp. is a Gram-negative, rod-shaped bacterium and Lactose-positive colonies cultivated on MacConkey agar.[13] *Klebsiella* spp. is the main bacterium of the family Enterobacteriaceae, which can produce Extended-spectrum β-lactamase (ESBL) to fight the β-lactam class of antibiotics, such as the Penicillin, Cephalosporin, Carbapenem, and Monobactam groups. This enzyme hydrolyzes the β-lactam ring from antibiotics so that antibiotic resistance can occur.[14]

Prophylactic antibiotics such as the Aminoglycosides and Cephalosporin first generation are often given in cases of open fractures to prevent infection. This is what allows antibiotic resistance.[15] Antibiotic resistance could occur in some ways, such as destroying antibiotics with the enzymes produced, improving antibiotic capture point receptors, improving the physicochemical targets of antibiotic targets in bacterial cells, and antibiotics could not penetrate bacterial cell walls due to changes in bacterial cell wall properties. If someone is infected with

resistant bacteria, the effort to deal with infection with antibiotics is more complicated.[16]

Research conducted at Voi County Hospital, Kenya, by Sitati et al. in 2017, stated that Gram-negative bacteria *Klebsiella* spp. and *Pseudomonas* spp. pre and post-debridement experienced high resistance to Tetracycline and Amoxicillin-Clavulanic Acid by 27% and 23% and experienced resistance of 87.5%, 91%, and 47.6% in Gentamicin, Amikacin, and Cefuroxime.[12]

It was giving Ceftriaxone therapy as a prophylactic antibiotic and Cefixime when outpatient to patients with open fractures in the emergency room of Emergency department dr. Soebandi hospital Jember is not based on culture results and antibiotic sensitivity testing. This is what underlies the author's research on the sensitivity of 12 types of antibiotics, namely Amoxicillin, Tetracycline, Ceftriaxone, Gentamicin, Cefixime, Ciprofloxacin, Penicillin, Meropenem, Erythromycin, Vancomycin, Cefadroxil, and Chloramphenicol against *Klebsiella* spp. in the post-debridement culture of cases of open fractures in emergency department dr. Soebandi hospital Jember.

## MATERIALS AND METHODS

### Materials

The population of this study was 12 bacterial isolates from the post-debridement culture of open fracture patients in the emergency room of emergency department dr. Soebandi hospital Jember from March to May 2019 consisted of *Klebsiella* spp. (5 patients), *Pseudomonas* spp. (3 patients), *Shigella* spp. (2 patients), *Salmonella* spp. (1 patient), and *Proteus* spp. (1 patient). The sample of this study was *Klebsiella* spp. amounting to 5. The 12 types of antibiotics, namely Amoxicillin, Tetracycline, Ceftriaxone, Gentamicin, Cefixime, Ciprofloxacin, Penicillin, Meropenem, Erythromycin, Vancomycin, Cefadroxil, and Chloramphenicol. The research used McFarland standards, Mueller-Hinton Agar, plates, sterile cotton swabs, aluminum foil, tweezers, syringes, caliper, and ruler.

### Methods

This study uses a laboratory explorative research design that is research that does not aim to look for relationships between variables, is only descriptive and is carried out at the Laboratory of Microbiology, Faculty of Medicine, University of Jember. The method used is diffusion (Kirby Baurer) by matching the inhibition zone diameters using the standard Clinical Laboratory Standards Institution (CLSI) table to determine sensitive, intermediate, or resistant. The steps of the research procedure was to prepare 0.5 McFarland standards made from 1% $BaCl_2$ and 1% $H_2SO_4$ and shake before use to adjust the turbidity of the bacterial suspension and Mueller-Hinton Agar from 15.2 grams of Mueller-Hinton and 400 ml aquadest.

Antibiotic sensitivity testing in this study used the method disc diffusion (Kirby-Baurer test) with Mueller-Hinton Agar. The steps taken were to make inoculums from *Klebsiella* spp. from each plate using a loop into 2 ml of NaCl 0,9%. The inoculum turbidity was adjusted to ensure an even or nearly even growth yield using McFarland standard. After turbidity obtained the same results as McFarland standard, the plate was inoculated using a sterile cotton swab dipped in the inoculum in laminar flow biobase.

Before being swabbed on Mueller-Hinton Agar, excess inoculum was removed by pressing and rotating the cotton swab firmly against the side of the tube. The swab was evenly distributed over the entire surface of Mueller-Hinton Agar by rotating the plate at an angle of 60˚ and allowed to dry for several minutes at room temperature with the cup closed. Then given 4 discs of antibiotics in each medium.

Discs are aseptically placed on the surface of Mueller-Hinton Agar using sterile tweezers to avoid contamination with other bacteria. The media that had been given an antibiotic disc was incubated for 24 hours at $37^0$C. Repetition was carried out three times on different media.

Measurements were made the next day after 24 hours of media incubation at $37^0$C. Measuring the diameter of the bacterial growth inhibition zone using a caliper or ruler is done on the back of the Mueller-Hinton Agar media so that you don't have to open the lid. The measurement results are adjusted to the Clinically and Laboratory Standards Institute (CLSI) in the classification of sensitive, intermediate, or resistant.

### Ethical Approval

This research received ethical approval from the health research ethics comitte of Faculty of Medicine, University of Jember with the letter number 1.408/H25.1.11/KE/2020.

## RESULTS AND DISCUSSION

Based on preliminary studies carried out from March to May 2019 at the IGD RSD, dr. Soebandi Jember, in 30 patients with open fractures, there were data that there were 12 patients in the culture of positive post-debridement growing bacteria, 42% *Klebsiella* spp., 25% *Pseudomonas* spp., 17% *Shigella* spp., 8% *Salmonella* spp., and 8% *Proteus* spp.. *Klebsiella* spp. is the most common bacteria in post-debridement culture, 5 isolates. Patients positive for *Klebsiella* spp. in the post-debridement culture there were 5 people with 4 male and 1 female. In contrast, the age distribution of patients was 1 person in range 17-25 years, 2 people in range 36–45 years, 1 person in range 56–65 years, and 1 person in range ≥ 66 years. All patients were diagnosed with varying degrees of open fracture according to the Gustilo-Anderson classification. Most patients experience open fractures due to traffic accidents. The clinical characteristics data of 5 patients with open fractures, such as diagnosis and Mode of Injury (MOI) (shown in Table 1).

**Table 1.** Diagnosis, Grade, and MOI of the Open Fractures Patients

| Patients | Diagnosis | Grade | Mode of Injury (MOI) |
|---|---|---|---|
| P1 | Traumatic amputation digiti 2, open fracture head metacarpal 2, and open fracture digiti 3 phalanx distal manus sinistra | IIIB | Exposed to a wood-cutting knife |
| P2 | Open fracture fibula dextra and fracture iliac wing dextra | IIIA | Traffic accidents between 2 motorcycle riders |
| P3 | Open fracture tibia-fibula 1/3 medial sinistra | IIIB | Traffic accidents between 2 motorcycle riders |
| P4 | Open fracture digiti 1,2,3 phalanx proximal pedis dextra | IIIA | Traffic accidents between 2 motorcycle riders |
| P5 | Open fracture kominutif tibia-fibula sinistra | II | Traffic accidents between 2 motorcycle riders |

The results of medical record data on the type of antibiotic prophylaxis, antibiotics during hospitalization, and antibiotics consumed at home used by patients with open fractures as a sample of this study can be seen in Table 2. The antibiotics tested were adjusted to the CLSI standard for *Klebsiella* spp. as shown in Table 3.

**Table 2.** Antibiotics Are Given to Patients with Open Fractures at dr. Soebandi Hospital

| Patients | Antibiotic | | |
|---|---|---|---|
| | **Prophylaxis** | **Inpatient** | **Outpatient** |
| P1 | *Ceftriaxone* | *Ceftriaxone* | *Cefixime* |
| P2 | *Ceftriaxone* | *Ceftriaxone* | *Cefixime* |
| P3 | *Ceftriaxone* | *Ceftriaxone* | *Cefixime* |
| P4 | *Ceftriaxone* | *Ceftriaxone* | *Cefixime* |
| P5 | *Ceftriaxone* | *Cefazoline and Gentamicin* | *Cefixime* |

**Table 2.** The Result of the Antibiotic Sensitivity Test for *Klebsiella* spp.

| Sample | AML | CRO | CFM | P | MEM | CFR | CN | CIP | TE | E | C | VA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P1 | R | R | R | R | R | R | R | I | R | I | I | S |
| P2 | R | R | R | R | R | R | S | I | I | R | S | S |
| P3 | R | R | R | R | R | R | R | I | R | I | I | S |
| P4 | R | R | R | R | R | R | R | S | I | I | I | S |
| P5 | R | R | R | R | R | R | R | I | S | I | I | S |

R= Resistent, I= Intermediate, S= Sensitive, P1= Patient 1, P2= Patient 2, P3= Patient 3, P4= Patient 4, P5= Patient 5, AML= *Amoxicillin*, CRO= *Ceftriaxone*, CFM= *Cefixime*, P= *Penicilin*, MEM= *Meropenem*, CFR= *Cefadroxil*, CN= *Gentamicin*, CIP= *Ciprofloxacin*, TE= *Tetracycline*, E= *Erythromycin*, C= *Chloramphenicol*, VA= *Vancomycin*.

This study found positive patients *Klebsiella* spp. in the post-debridement culture. There were 5 people, 4 male, and 1 female, with an age range of 22-70 years. The results of this study are consistent with research by Agarwal et al[2], which states that of the 70 open fracture patients studied, 63 patients (90%) were men with ages ranging from 3-75 years.[2] Research by Gupta et al[10] states that most open fracture patients have a 13 times higher incidence of males than females. This is because men are more often outdoors activities.[10] Traffic accidents are the most common cause of open fractures, followed by work-related injuries and falls from heights. High-energy trauma is the most common Mode of Injury (MOI) causing open fractures. Diagnosis of patients with open fractures dominated by phalanx, tibia, and fibula according to research by Sop and Sop[4], which states that phalanx fractures are the most common fractures, followed by tibia and fibula fractures.[4] Degree III open fractures, according to the Gustilo-Anderson classification, have a significantly higher infection rate than grades I and II. This is related to the severity of the wound and the degree III treatment's length of time.[17–19]

Management of open fracture cases requires the administration of antibiotic prophylaxis to prevent surgical site infection (SSI). Prophylactic antibiotics used in treating open fractures in RSD dr. Soebandi Jember uses the Ceftriaxone antibiotic, while the antibiotic given while outpatient is Cefixime. Ceftriaxone and Cefixime are included in the antibiotic. A cephalosporin is an antibiotic option in line with guidelines for treating open fractures in a journal by Zalavras[20]. The journal also describes the administration of Cephalosporin and Aminoglycosides antibiotics to prevent infection. Cephalosporin is given to prevent Gram-positive bacteria in first and second-degree open fractures. In contrast, third-degree open fractures require antibiotics to protect against Gram-positive and negative, and Aminoglycosides (Gentamicin) are given.[20] According to Sop and Sop[4], when antibiotics are given 66 minutes after injury, the infection rate is 0% and increases to 17% if it exceeds this time.[4] The British Orthopedic Association/British Association of Plastic Reconstruction and Aesthetic Surgeons (BOA/BAPRAS) supports the opinion of experts that antibiotics are given

24 to 48 hours for the degree I and a maximum of 72 hours in degrees II and III.[21]

In this study, a sensitivity test for 12 antibiotics was carried out on the *Klebsiella* spp. These bacteria can cause many of disease, cause problem to people with immunocompromised and the most common cause of hospital acquired pneumonia. In this study *Klebsiella* spp. that found in culture most likely caused of open fracture, even the management has been carried out according to the procedure.[22,23] Open fracture treatment depends on location of fracture but generally need irrigation, debridement, and antibiotic. Initial debridement in should be performed within 24 hours. The goals of open fracture management include decreasing risk of infection and promoting fracture union.[24,25]

*Klebsiella* spp. cultured after debridement in patients with open fractures developed resistance to β-lactam antibiotics. These antibiotics include amoxicillin, Ceftriaxone, Cefixime, Penicillin, Meropenem, and Cefadroxil. However, antibiotics such as Gentamicin, Ciprofloxacin, Tetracycline, Chloramphenicol, and Vancomycin, these bacteria are sensitive. Associated with the resistance of these bacteria to β-lactam class antibiotics, it proves that these bacteria are Extended-spectrum β-lactamase (ESBL) producing bacteria.

The results of this study different from studies conducted at Voi County Hospital, Kenya by Sitati et al[12] which states that patients with open fractures after culture in pre and post debridement, obtained high resistance data against Tetracycline, Erythromycin, and Amoxicillin-Clavulanic Acid in Gram positive and negative bacteria. Gram negative bacteria such as *Klebsiella* spp. and *Pseudomonas* spp. found in pre and post debridement experienced high resistance to Tetracycline and Amoxicillin-Clavulanic Acid by 27% and 23% and experienced resistance of 87.5%, 91%, and 47.6% in Gentamicin, Amikacin and Cefuroxime. This is likely due to differences in study locations and differences in the selection of antibiotics used in the treatment of open fracture patients.[12,27] The results of research that have been done have shown that antibiotics are still sensitive to *Klebsiella* spp. namely Gentamicin in 1 patient, Ciprofloxacin in 1 patient, Tetracycline in 1 patient, Chloramphenicol in 1 patient, and Vancomycin in all patients. ESBL-producing bacteria can be given Vancomycin antibiotics which can kill bacteria by breaking peptide bonds between amino acids in the peptidoglycan wall. Although using Vancomycin for open fractures is safe, it is still controversial, except for patients who are allergic to Penicillin. This is because Vancomycin added to Cefazoline has no benefit in patients with open fractures.

However, a recent 2016 publication by Tennent et al shows the benefits of using Vancomycin powder in local wounds of rats to prevent biofilm formation.[28] Gentamicin antibiotics were still sensitive according to the study of Ashwin and Thomas[29] , which stated that bacterial culture in third-degree open fractures was 83.3% *Klebsiella* spp. sensitive to Gentamicin.[29]

Chloramphenicol antibiotics are sensitive to *Klebsiella* spp. This is in accordance with research by Nitzan et al[30] which states that members of Enterobactericeae, such as the bacteria *Klebsiella* spp., *E. coli*, and *Enterobacter* spp. achieve statistical significance of a lower level of resistance to Chloramphenicol of 18.4%.[16] Ciprofloxacin antibiotics are sensitive to *Klebsiella* spp. by the 2018 Mangala study, which states that Ciprofloxacin has a 69% sensitivity to the Enterobacteriaceae family.[31]

Research on Carbapenem-Resistant Klebsiella Pneumoniae (CRKP) states that in data analysis from 1998–2010, resistance to Tetracycline increased only slightly. Next-generation Tetracycline may be helpful in the treatment of CRKP because of increased tissue penetration, antibiotic activity, and the decreased tendency for antibiotic resistance.[32] This is in line with research that has been done because the Tetracycline

antibiotic was found to be sensitive in 1 patient. Erythromycin antibiotics were concluded intermediates against the bacteria *Klebsiella* spp. because intermediates were obtained in 4 patients and resistant in 1 patient. Research by Khan et al[33] states that the bacteria *Klebsiella* spp. found to be more resistant to macrolide antibiotics, equal to 41.67% against Erythromycin.[33] resistant Antibiotics and intermediates cause the treatment of open fractures to be suboptimal, resulting in surgical site infection (SSI). The management of open fractures is focused on effective debridement measures, appropriate antibiotic therapy, and initial wound closure to prevent infection.[21]

The older age (71.5%) due to experiencing immune deficiency (decreased immune system) resulting in a longer recovery time. Degree III open fractures according to the Gustilo-Anderson classification have a significantly higher infection rate than grades I and II. This is related to the severity of the wound and the length of time of the third degree treatment.[13] The occurrence of SSI in these patients is likely due to the above SSI risk factors, such as age 70 years (> 60 years), male, experiencing high energy injuries due to accidents, and the degree of open fracture IIIB.

## CONCLUSIONS

The conclusion of this study after an antibiotic sensitivity test was conducted on 5 samples of *Klebsiella* spp. in post-debridement culture in Emergency Department Soebandi General Hospital Jember is resistant to *Klebsiella* spp. the β-lactam class of antibiotics used in this study are Amoxicillin, Ceftriaxone, Cefixime, Penicillin, Meropenem, and Cefadroxil. *Klebsiella* spp. was still sensitive to other antibiotics such as Chloramphenicol Gentamicin, Ciprofloxacin, Tetracycline, and Vancomycin. Erythromycin antibiotics are stated intermediates to the bacterium *Klebsiella* spp. for the dr. Soebandi hospital Jember. The institution needs to have periodic culture tests and antibiotic sensitivity tests for inpatients so that an antibiogram can be made and used as a basis for the definitive treatment of diseases, especially in the field of Orthopedic.

The antibiotic sensitivity test towards *Klebsiella* spp. which contaminated the post-debridement procedure in patients with open fracture, showed an evidence that a comprehensive evaluation of the empirical antibiotic prophylactic strategies pre- and post-operative procedures so far need to be considered. This statement is based on the total resistance of Ceftriaxone and Cefixime in all *Klebsiella* spp samples. Based on this research, we also humbly recommend other antibiotics that can be alternative options as prophylactic agents to prevent perioperative contamination and postoperative surgical site infection in patients with open fractures, such as Gentamicin, Ciprofloxacin, Tetracycline, Chloramphenicol, and Vancomycin.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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***Review Article***

# Prolonged Use of Protective Masks Induced Facial Skin Injury in Primary Healthcare Workers during COVID-19 Pandemic: A Systematic Review

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***ABSTRACT***

*COVID-19 transmission necessitates health workers to use personal protective equipment (PPE), especially protective masks when delivering medical services. Long-term use of protective masks might cause facial skin injuries. Our study aims to provide a systematic review to explore the phenomenon and incidence of protective masks induced facial skin injuries in primary healthcare workers. This systematic review was created by obtaining articles from the PubMed database and the Cochrane library from 2020 to 2021, using the keywords "Face skin injury," "Wearing protective masks for a long time," and "Wearing protective masks and facial skin disorders." Inclusion criteria were studies that fully report the phenomenon of wearing protective masks and the incidence of facial skin injuries. One hundred and sixty-eight studies were obtained, but only 14 articles matched the inclusion criteria with more than 10,430 participants from different countries that covered various characteristics of facial skin injuries in primary healthcare workers. The findings obtained dominant characteristics of health workers who experienced facial skin injuries: women, N95 masks, and daily N95 coverage for more than 6 hours ($p<0.05$). Facial skin injuries are often seen after using protective face masks, as it is used for an extended period as part of a defensive effort during work. Therefore, measures that protect health workers from COVID-19 and prevent health workers from potential injuries of protective masks must be taken into account.*

***Keywords:*** *COVID-19; facial skin injury; long duration; primary health workers; protective masks*

***ABSTRAK***

*Penularan COVID-19 mengharuskan tenaga kesehatan untuk menggunakan alat pelindung diri (APD), khususnya masker pelindung saat memberikan pelayanan medis. Penggunaan masker pelindung jangka panjang dapat menyebabkan cedera kulit wajah. Penelitian kami bertujuan untuk memberikan tinjauan sistematis untuk mengeksplorasi fenomena dan kejadian cedera kulit wajah terkait penggunaan masker pelindung pada tenaga kesehatan primer. Tinjauan sistematis ini dibuat dengan memperoleh artikel dari database PubMed dan perpustakaan Cochrane dari tahun 2020 hingga 2021, menggunakan kata kunci "Face skin injury," "Wearing protective masks for a long time," dan "Wearing protective masks and facial skin disorders". Kriteria inklusi adalah penelitian yang secara lengkap melaporkan fenomena pemakaian masker pelindung dengan kejadian luka pada kulit wajah. 168 penelitian diperoleh, tetapi hanya 14 artikel yang sesuai dengan kriteria inklusi dengan lebih dari 10.430 peserta dari berbagai negara yang mencakup berbagai karakteristik cedera kulit wajah pada petugas kesehatan primer. Temuan didapatkan karakteristik dominan tenaga kesehatan yang mengalami cedera kulit wajah: perempuan, masker N95, dan cakupan N95 harian lebih dari 6 jam ($p<0,05$). Cedera kulit wajah sering terlihat setelah menggunakan masker pelindung, karena digunakan dalam*

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*waktu lama sebagai bagian dari upaya defensif selama bekerja. Oleh karena itu, langkah-langkah yang melindungi petugas kesehatan dari COVID-19 dan mencegah petugas kesehatan dari potensi cedera masker pelindung harus diperhitungkan.*

***Kata kunci:*** *COVID-19; cedera kulit wajah; durasi panjang; masker pelindung; petugas kesehatan layanan primer*

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## INTRODUCTION

Coronavirus disease (COVID-19) is an ongoing global threat requiring the public to abate its transmission by improving personal and communal hygiene practices.[1,2] Personal Protective Equipment (PPE) is essential for health workers as they are more at risk of contracting COVID-19.[3-5]

Although wearing PPE, especially protective masks, is mandatory to prevent COVID-19 infection, its long-term use increases the temperature, which leads to sebum excretion. Moreover, the pressure and friction from the protective masks can cause contact dermatitis (injuries of facial skin), seborrheic dermatitis, and acne vulgaris. The most frequent side effect of PPE is pressure-based wounds induced by N95 masks, such as the indentation of the mask on the bridge of the nose of health workers.[5]

This systematic review will provide a comprehensive overview of the available literature regarding the side effects of the long-term use of protective masks. Our main objective is to understand the extent of facial skin injury induced by protective mask-wearing among primary healthcare workers during the Pandemic of COVID-19.

## METHODS

### Study Design

This was a systematic review of facial skin injury induced by protective masks during the COVID-19 Pandemic. In conducting the literature search and reviewing the article, we adhered to PRISMA guidelines.[4]

PubMed and Cochrane library were the primary databases to search for articles published from January 2020 to November 2021. The literature search process used the Boolean operator "AND" or "OR" using the keywords "Face skin injury," "Wearing protective masks for a long time," and "Wearing protective masks and facial skin disorders."

### Study Selection

Articles were selected from the databases based on inclusion and exclusion criteria. The article's inclusion process followed several criteria, such as 1) Studies reporting the significance of protective masks induced facial skin injury during the Pandemic of COVID-19; 2) Age > 18 years old; 3) Medical staff who wore level 2 or 3 PPE while working at the frontline against COVID-19, regardless of gender. Exclusion criteria included review articles written in languages other than English, conference abstracts, nonhuman research, and studies that did not evaluate the outcome measures.

Two independent reviewers selected the articles and extracted the key findings. Disagreements between the two authors were resolved by reaching a consensus aided by the third reviewer. The full literature search and selection process followed the PRISMA Guideline.

### Study Quality

Assessing the quality of evidence within a systematic review is as important as analyzing the data. Selecting an appropriate tool to help analyze strength of evidence and embedded biases within each paper was also essential. Therefore, the author used Joanna Briggs Institute (JBI) that provides robust

checklists for the appraisal and assessment of most studies.

### Data Extraction and Analysis

Key findings were independently extracted, starting by noting baseline characteristics and outcomes from included articles. Extracted data contained first author name, year of publication, study design, age range, diagnosis, sample size, and results. All data results are presented and described descriptively in tabular form.

## RESULTS AND DISCUSSION

The querying process returned 168 studies, with 167 originating from online databases (PubMed and Cochrane library) and one article sourced from an organic search. A total of 123 studies were obtained after removing duplicates using computer software (Citation Manager). Upon screening the title and abstract, 17 studies were eligible for further assessment. However, 3 studies did not satisfy the inclusion criteria, 14 of which were still included in the qualitative analysis (systematic study).[5–18] Figure 1 summarizes the literature search process as indicated by the PRISMA Guideline.

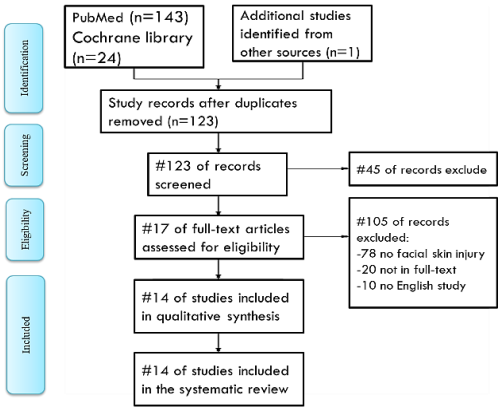


**Figure 1.** Flowchart PRISMA

From all included articles, 10,430 respondents participated in several observational studies. The median age of respondents was 35 years, with most respondents being women (available in Table 1).

The dominant health workers are nurses with the most use of N95 while handling patients during a pandemic. In addition, the working time of health workers in each study was between 4–12 hours.

**Table 1.** Characteristics of Studies

| Author, year | Study design | Age | Gender | Sample size |
|---|---|---|---|---|
| Jiang *et al*., 2020[5] | Multicenter observational study | 35 years (median) | Male (12.7%)<br>Female (87.3%) | 4308 |
| Battista *et al*., 2021[6] | Observational study | 35.0 ±11.7 years | Male (33.1%)<br>Female (66.9%) | 381 |
| Abiakam *et. al*., 2021[7] | Prospective study | 45 years (median) | Male (12.0%)<br>Female (88.0%) | 307 |
| Ippolito *et al*., 2021[8] | A cross-sectional survey | 40 years (median) | Male (49%)<br>Female (51%) | 2711 |
| Han *et al*., 2021[9] | A cross-sectional study | 37.5±10.83 years | Male (10.0%)<br>Female (90.0%) | 20 |
| Choi *et al*., 2021[10] | Multicenter observational study | 35.50±14.45 years | Male (34.85%)<br>Female (65.15%) | 330 |
| Uthayakumar *et al*., 2021[11] | Rapid report | 34 years (median); Range 23-60 | Female : male (4:1) | 67 |
| Purushothaman *et al*., 2021[12] | Cross-sectional | 25.843 years (mean)<br>Range 20-48 | Male (28.4%)<br>Female (71.6%) | 250 |
| Techasatian *et al*., 2020[13] | Prospective Cross-sectional | 32 (25-41) years (median (IQR))<br>Range 18-87 years | Male (26.7%)<br>Female (73.3%) | 833 |
| Singh *et al*., 2020[14] | Survey study | 32.78±14.51 years | Male (59.7%)<br>Female (40.3%) | 43 |
| Coelho *et al*., 2020[15] | Cross-sectional | 34.08 (8.9) (mean(SD)) | Male (16.4%)<br>Female (83.6%) | 1106 |
| Yuan *et al*., 2020[16] | Cross-sectional | N/A | Male : Female (1:2) | 129 |
| Shanshal *et al*., 2020[17] | Cross-sectional observational | N/A | Male (36%)<br>Female (64%) | 276 |
| Christopher *et al*., 2020[18] | Cross-sectional | 26.94±7.23 years | Male (33%)<br>Female (67%) | 200 |

Personal protective equipment (PPE) is one piece of equipment used by health workers to prevent nosocomial infections and protect patients from the possibility of infection, starting from the patient entering and receiving healthcare and medical action until the patient returns from the hospital.[19–22]

The scientific summary released by the World Health Organization (WHO) reported the presence of SARS-CoV-2 ribonucleic acid (RNA) in air samples taken from under the patient's bed and windows. Both areas would have minimal direct contact with patients or health care. Researchers also found that 66.7% of air samples taken from hospital hallways contained viral.[23,24]

The World Health Organization (WHO) recommendation is that surgical masks should be sufficient when treating COVID-19 patients, and N95 or PAPR respirators should be used only in the case of aerosol-generating procedures. The CDC insists that N95 respirators be used by all medical professionals who contact COVID-19 patients. Based on this, if there are difficulties in procurement or vacancies for N95 masks, surgical masks are allowed to make contact with COVID-19 patients and to protect, face shields can be used. Several studies state no clinically significant evidence of a difference in safety between surgical masks and N95.[5,8,20]

**Table 2.** Unique Publications Identified

| Author, year | PPE and Duration | Outcome | Quality of Study (Score) |
|---|---|---|---|
| Jiang *et al*., 2020[5] | Level 3 PPE, protective masks >4 hours | The device-related pressure injury (DRPI) was prevalent among healthcare workers wearing PPE against COVID-19. The risk factors for facial skin injury ($p<0.05$) were male, wearing level 3 PPE, longer wearing time > 4 hours and sweating. | High (8) |
| Battista *et al*., 2021[6] | Surgical Mask, Cotton Mask, N95, Combination Surgical + FFP2/3, <1 hours until > 12 hours | Most affected individuals were healthcare workers wearing N95 respirator masks for more than six h/d ($p<0.05$) | Moderate (6) |
| Abiakam *et al*., 2021[7] | PPE (FFP3), eye protection, gloves, gown >8 hours | The adverse skin reactions (facial skin injury) had a significant association with the average daily time of PPE usage during > 8 hours ($p<0.05$) | Moderate (7) |
| Ippolito *et al*., 2021[8] | Mask (Surgical, N95, FFP3, PAPR), Gown, >6 hours | 59% of the participants had significant pressure injury on the face area after using an N95 mask in ICU for> 6 hours ($p<0.05$) | High (8) |
| Han *et al*., 2021[9] | KF94 respirator dan medical mask 4 hours, 8 hours, dan 14 hours | Skin injury significantly differed between RPE-covered and uncovered areas after 4 and 8 hours ($p<0.05$). | Low (2) |
| Choi *et al*., 2021[10] | N95/KF94/KF80, Surgical, Cotton ≥6 hours | Daily use of N95 masks significantly increases the incidence of new contact dermatitis. The duration of wearing PPE >6 hours/day and masks made of cotton significantly increased the incidence of acne and wounds around the face. Health workers had a higher incidence of facial skin injuries ($p<0.05$). | Moderate (6) |
| Uthayakumar *et al*., 2021[11] | Protective masks N95 > 6 hours | PPE marked an increase in the impact of facial skin injury; 70% reported a significant adverse effect on their work or study ($P<0.05$) | Low (4) |
| Purushothaman *et al*., 2021[12] | N95 + surgical mask, > 4 hour/day | Excessive sweating around the mouth after used protective mask was 67.6%, resulting in poorer adherence and increased risk of infection in the face area ($p<0.05$). | Moderate (7) |
| Techasatian *et al*., 2020[13] | N95 masks, surgical mask, 4 to 8 hours/day | 1,92% facial skin injury among 4-8 hours (48.9%) after used protective mask was a significant value in statistics ($p<0.05$) | High (8) |
| Singh *et al*., 2020[14] | N95 masks, face shields, and goggles Average 8.76 hours | Goggles and N95 masks were the most common culprit agent among all PPE, causing skin injuries. The most commonly noted dermatoses were irritant contact dermatitis in the face ($p<0.05$). | Moderate (7) |
| Coelho *et al*. 2020[15] | Cap, gloves, apron, N95 mask, surgical mask, PFF2 mask, face protector, and glasses >6 hours | The number of pressure injuries related to personal protective equipment was high (an average of 2.4 injuries per professional). Working and wearing personal protective equipment for more than six hours a day was one of the significant factors ($p<0.05$). | High (8) |

| | | | |
|---|---|---|---|
| Yuan *et al*. 2020[16] | N95 mask, goggles, gloves, face mask, gown, and medical protective clothing > 8 hours | A total of 122 (94.57%) healthcare professionals experienced discomfort while wearing L3PPE, including varying degrees of face skin injuries, respiratory difficulties, heat stress, dizziness and nausea. | Moderate (6) |
| Shanshal *et al*. 2020[17] | N95 mask, goggles, gloves, face mask, gown, and medical protective clothing > 8 hours | 51% had pressure injury in the facial skin after prolonging (> 8 hours) using PPE, especially in the woman, and 82.5% had facial skin injury ($p<0.05$) | Moderate (6) |
| Christopher *et al*. 2020[18] | Level 1-3 PPE, protective masks ≥7 hours/day | The level of PPE worn and duration of PPE worn daily was factors considerably associated with adverse skin reactions to PPE. | Low (4) |

FSI=Facial Skin Injury, manifested in several clinical features, such as dryness, itching, erythema, acne, indentation, and pressure ulcer.

Further evidence suggested N95 respirator as protective mask causes more severe facial injuries than the KN95 respirator.[5] Applying polyester tape layering and emollient effectively prevented severe injuries, especially on the cheekbones, chin, nasal bridge and behind the ears.[25–29]

N95 masks cause skin injury because the material is thick and stiff, causing greater pressure on the skin.[9] Also, many studies have reported differences in risk between N95 masks and KN95 masks, as observed in our results. The difference in risk is interesting, given that N95 and KN95 masks provide relatively the same level of protection. More recent tests have also shown that N95 and KN95 are quite effective at filtering respiratory particulates, especially those protective mask used by healthcare professionals in treating patients with COVID-19. Besides that, interestingly, the KN95 mask is not as thick and stiff as the N95, so it is more comfortable to use for a longer period.[7,8,30,31]

The quality of the study and the bias assessment of the cross-sectional studies was done using the Newcastle Ottawa Scale (NOS), as presented in Table 2. The overall quality of evidence was moderate-high quality.[4,23] Our findings recommend using an alternative to KN95 masks instead of N95 in primary care for patients with COVID-19. They can promote using wound dressings and emollients to protect facial skin after carrying out services with PPE for > 4–6 hours. In particular, healthcare facilities are expected to provide supplies of protective facial mask and emollients to prevent facial injuries that use PPE too often and for a long time.[32,33]

Previous investigations have yielded similar conclusions, although this study is one of the few to report the phenomenon of facial injuries due to prolonged use of protective masks. These results can be considered, and recommendations can be used in Indonesia wisely. However, much remains to be learned about the COVID-19 Pandemic on the welfare and safety of health workers in primary health care. Future studies should explore minimal treatment and prevention options for healthcare workers who suffer these injuries so that services during the Pandemic are maximized.[34,35]

## SUMMARY

Facial skin injuries are often seen after using protective masks, as it is used for an extended period of defensive effort during work. The current state of the evidence suggests that some protective face mask have their respective advantages and optimal usage duration. Therefore, measures that protect health workers from COVID-19 and prevent health workers from potential injuries from protective facial masks must be considered. The choice and duration of protective mask usage must be adjusted according to their working environment.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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***Original Article***

# Epidemiology of *Escherichia coli* as a Critical Pathogen of Bloodstream Infection Patients in Dr. Soetomo General Hospital, Surabaya, Indonesia

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***ABSTRACT***

*Bloodstream infections (BSI), caused primarily by multidrug-resistant Escherichia coli, are a significant cause of morbidity and mortality worldwide. This study aims to evaluate the epidemiology of E. coli as a critical pathogen in patients with bloodstream infections in a tertiary referral hospital. This is a retrospective study using a descriptive observational research design. This study used a medical record instrument for bloodstream patients in Dr. Soetomo Hospital's inpatient ward with Gram-negative bacteria results of blood cultures in the Clinical Microbiology Laboratory from April 2021 to September 2021. The observed variables include; antimicrobial sensitivity, patient clinical characteristics, demographic data, clinical diagnosis, and clinical outcome. In 6 months, 276 Gram-negative bloodstream infection patients were treated at Dr. Soetomo Hospital. The proportion of E. coli was 17 %. The main characteristics of patients were over 60 years old (28%), and 54% were female. 63% of E. coli were ESBL, and 9% were carbapenem-resistant microorganisms. High antimicrobial resistance was found in quinolones (100%), ampicillin (93%), piperacillin (74%), tetracycline (72%), ceftriaxone (66%), cefotaxime (65%), ceftazidime (60%), cefazolin (65%), and trimethoprim-sulfamethoxazole (65%). The most common potential determinant profile discovered was linked to immunocompromised status due to malignancy. The high number of antimicrobial-resistant bacteria showed the importance of strict infection control and updated epidemiology data as a guide for empirical antimicrobial therapy.*

***Keywords:*** *bloodstream infection; E.coli; epidemiology; ESBL; resistance*

***ABSTRAK***

*Infeksi aliran darah (IAD), yang terutama disebabkan oleh Escherichia coli yang bersifat multi-drug resistance microorganisms (MDRO), merupakan penyebab signifikan morbiditas dan mortalitas di seluruh dunia. Penelitian ini bertujuan untuk mengevaluasi epidemiologi E. coli sebagai patogen pada pasien infeksi aliran darah di rumah sakit rujukan tersier. Penelitian ini merupakan penelitian deskriptif dengan desain penelitian observasional menggunakan alat rekam medis aliran darah pasien di ruang rawat inap RSUD Dr. Soetomo dengan bakteri Gram negatif hasil kultur darah di Laboratorium Mikrobiologi Klinik pada bulan April 2021 sampai September 2021. Variabel yang diamati meliputi; sensitivitas antimikroba, karakteristik klinis pasien, data demografis, diagnosis klinis, dan hasil klinis. Dalam 6 bulan, didapatkan 276 pasien infeksi aliran darah Gram-negatif dirawat di RS Dr. Soetomo. Proporsi E. coli adalah 17%. Karakteristik utama pasien berusia di atas 60 tahun (28%), dan 54% berjenis kelamin perempuan. 63% E. coli adalah ESBL, dan 9% adalah mikroorganisme yang resisten terhadap karbapenem. Resistensi antimikroba yang tinggi ditemukan pada kuinolon (100%), ampisilin (93%), piperacillin (74%), tetrasiklin*

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*(72%), ceftriaxone (66%), cefotaxime (65%), ceftazidime (60%), cefazolin (65%), dan trimethoprim-sulfamethoxazole (65%). Profil penentu potensial yang paling umum ditemukan terkait dengan status immunocompromised karena keganasan. Tingginya jumlah bakteri resisten antimikroba menunjukkan pentingnya pengendalian infeksi yang ketat dan data epidemiologi terkini sebagai panduan terapi antimikroba empiris.*

***Kata kunci:*** *E. coli; epidemiologi; ESBL; infeksi aliran darah; resistensi*

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## INTRODUCTION

Bloodstream infection (BSI) is a big challenge of infectious diseases. It represents 40% of community-acquired (CA) cases, hospital-acquired (HA) sepsis and septic shock, and approximately 20% of ICU-acquired cases.[1] It is invariably associated with poor outcomes significantly when adequate antimicrobial therapy and source control are delayed.[2] The pathogens causing bloodstream infection majority caused by Gram-negative bacteria, including *E. coli*.[3, 4] *E. coli* is a bacteria that often has resistant mechanisms to multiple antibiotics. These bacteria have built-in resistance mechanisms and can pass on genetic material that allows other bacteria to become drug-resistant. Because of this, *E. coli* was covered as a critical pathogen by the WHO in 2017.[5,6] BSI caused by multi-drug resistant (MDR) organism make the management difficult because the antibiotic therapy is limited and unsuitable empirical antibiotic treatment is given. BSI by MDR *E. coli* was associated with poorer outcomes and a higher overall mortality rate.[7]

*E. coli*, including Extended-spectrum beta-lactamase (ESBL) producing and Carbapenem-resistant, can cause severe and frequently lethal infections, especially bloodstream infections (BSIs).[4,8,9] ESBLs-producing bacteria can hydrolyze broad-spectrum cephalosporins, monobactams, and penicillins, while Carbapenem-resistant is an *E. coli* isolate resistant to ertapenem, imipenem, meropenem, or any carbapenem antimicrobial.[10] Bloodstream infection caused by those organisms represents a challenge due to the limitation of antimicrobials as a drug of choice; furthermore, it can cause significant morbidity and mortality.

The critical pathogens in bloodstream infection are majority caused by Gram-negative bacteria, the most frequent pathogen was *E. coli*[3,4] which could be characteristically different profiles in various hospitals or patient care units. In a hospital setting, it is crucial to evaluate and monitor the updated epidemiology of causative agents of infection due to the prevention and infection control program and the updated empirical antibiotics in the hospital. Therefore, epidemiological studies on microorganism infection must be updated periodically. This research focuses on local epidemiology data of *E. coli* as a pathogen detected in bloodstream infection, including the resistance pattern and the determinants factor related to invasive devices and immunocompromised conditions.

## MATERIALS AND METHODS

### Materials

We used data from the records of blood culture results from the Clinical Microbiology Unit and medical records of patients with Gram-negative bloodstream infection in inpatient wards of Dr. Soetomo Hospital from April 2021 until September 2021. Ethical clearance from the ethics committee has been obtained by number 0660/LOE/301.4.2/ X/2021.

### Methods

This research is descriptive research. All medical records containing *Escherichia coli*

detected, antimicrobial sensitivity, and other determinant factors, i.e., patient clinical characteristics, demographic data, clinical diagnosis, history of invasive devices, antibiotic use, and clinical outcomes. In addition, species identification, antimicrobial susceptibility testing, and determination of resistant patterns, including ESBL-producing strains and Carbapenem-resistant strains, using BD BACTEC™ blood culture system and BD Phoenix™ system. Antimicrobial sensitivity interpreted based on Clinical Laboratory and Standards Institute (CLSI) guideline 2021. Statistical Analysis Data were analyzed with Microsoft Excel and presented in a frequency table with the percentage of each variable which was then converted into a descriptive form.

## RESULTS AND DISCUSSION

There were 276 Gram-negative bacteria of a total of 973 (28.4%) positive blood cultures of hospitalized patients in Dr. Soetomo Hospital Surabaya within 6 months. *E. coli* was found in 48 patients of 276 Gram-negative bacteria (17%). It can be seen that *E. coli* was the third rank of Gram-negative bacteria causing bloodstream infection (Table 1). Forty-three of the 48 patients with *E. coli* bloodstream infection with the complete medical record were analyzed.

**Table 1.** Distribution of Gram-Negative Bacteria Detected of Bloodstream Infection in Dr. Soetomo Hospital, Surabaya, from April 2021 – September 2021

| Gram-Negative Bacteria | n (%) |
|---|---|
| *Acinetobacter baumannii/calcoaceticus complex* | 67 (24) |
| *Klebsiella pneumoniae* | 63 (23) |
| *Escherichia coli* | 48 (17) |
| *Pseudomonas aeruginosa* | 22 (8) |
| *Enterobacter cloacae* | 19 (7) |
| *Other Gram-negative bacteria* | 57 (21) |
| **Total** | **276 (100)** |

This study's results align with the surveillance study about the trend of bloodstream infection in the USA reported that the most prevalent Gram-negative bacteria causing bacteremia from 2005 until 2016 was *E. coli*, with an incidence range of 20-24%.[3] In comparison, *E. coli* also was found to be the most prevalent pathogen (32.8% of cases), followed by *Staphylococcus aureus* (20.6%), *Klebsiella pneumoniae* (16.1%), and *Pseudomonas aeruginosa* (11.6%), in a study of bloodstream infections at a major teaching hospital in Rome within 9 years period, according to Angelis et al.[4]

Another data of 382 BSI cases in a tertiary teaching hospital ICU revealed the most frequently isolated microorganisms to be *Klebsiella pneumoniae* (11.52%), followed by *Escherichia coli* (9.95%).[11] Furthermore study about the profile of blood culture of sepsis patients in the Intensive Care Unit (ICU) – Dr. Soetomo Hospital Surabaya revealed that Gram-negative bacteria were 25% of the total positive culture. Of the Gram-negative bacteria, Enterobacteriaceae showed a proportion of 59%, followed by *Acinetobacter baumannii* at 29%.[12]

The 43 *E. coli* strains isolated from the blood culture of BSI comprised 20 male patients and 23 females. This data aligns with a systematic literature review report that women were more likely than men to develop *E. coli* bacteremia overall. According to age group stratification, this connection was only present in young and middle-aged individuals; in adults over 60, the incidence rates for men and women were comparable.[13]

The age of the patients showed in Table 2, where the subjects were between 0.01 years (neonates) to 81 years, with the most age distribution being over 60 years, namely 12 patients. This data is in accordance with a systematic literature review report that the incidence rate considerably rose with age. With estimated rates of 110, 154, and 319 episodes per 100,000 person-years among those aged 60 to 69, 70 to 79, and 80 years and older, respectively, older individuals' incidence rates were higher than the population norm.[13]

**Table 2.** Age Distribution of Patients with Bloodstream Infection Caused by *E. coli*

| Age Group (year) | n (%) |
|---|---|
| 0–10 | 6 (14) |
| 11–20 | 5 (12) |
| 21–30 | 1 (2) |
| 31–40 | 3 (7) |
| 41–50 | 7 (16) |
| 51–60 | 9 (21) |
| >60 | 12 (28) |

Out of 43 patients, 16 (37%) were referred from other hospitals (Table. 3); this could be associated with the role of Dr. Soetomo hospital as the tertiary referral hospital. The primary diagnoses of patients in this study were grouped into several criteria, namely malignancy, coronavirus infection, primary infection other than BSIs, bile duct atresia, and other diagnoses composed of the small proportion of diagnoses listed in the footnotes of the table. The primary disease diagnosis was malignancy in 12 patients (28%), coronavirus infection in 11 patients (26%), primary infection other than BSIs in 8 patients (19%), bile duct atresia in 3 patients (7%), and others in 9 patients (21%). The most significant proportion of patients with primary diagnoses were malignancy and coronavirus infections.

**Table 3**. Characteristics of the Patients in the Study

| Characteristics (n=43) | Patients (n/%) |
|---|---|
| Age (year; means, min-max) | 43; 0.01–81 |
| Gender (M/F) | 20 (46)/23 (54) |
| Referral patients | 16 (37) |
| Diagnosis for hospitalization | |
| Malignancy* | 12 (28) |
| Coronavirus infection | 11 (26) |
| Infection ** | 8 (19) |
| Bile duct atresia | 3 (7) |
| Other *** | 9 (21) |
| Nasogastric tube | 27 (63) |
| Ventilator/Intubation | 19 (44) |
| Surgery | 14 (33) |
| Using a central venous catheter (CVC) | 14 (33) |
| Immunosuppressant therapy in 30 days | 13 (30) |
| Total of patients | 43 (100) |

*acute myeloid leukemia, acute lymphocytic leukemia, anaplastic anemia, Non-Hodgkin's lymphoma, malignant neoplasm of the placenta, malignant neoplasm of the ovary, malignant neoplasm of the cervix, malignant neoplasm of uteri, malignant neoplasm of the bile duct, malignant neoplasm of the pancreas

**septicemia, abscess of the liver, acute pancreatitis, pneumonia, cholecystitis, acute peritonitis, intestines tuberculosis, congenital pneumonia

***myelodysplastic syndrome, myasthenia gravis, morbidly adherent placenta, other and unspecified ovarian cysts, congenital hydronephrosis, acute renal failure, communicating hydrocephalus, burn multi regions

Several determinants were recorded, including invasive devices and others that may be associated with bacteremia (Table 3). The data showed the use of invasive devices was nasogastric tubes (63%), ventilators/ intubation (44%), and the central venous catheter (CVC) (33%). Furthermore, we found surgery cases (33%), immunosuppressant therapy (30%), and neutropenia (16%). A systematic literature review concluded that central and peripheral venous catheters increased the risk of *E. coli* bacteremia: by 10-fold and 7.5-fold, respectively. In contrast, suprapubic and urethral urinary catheters increased the risk by 6-fold and 3-fold, respectively.[13]

The proportion of patients with malignancy was relatively high, namely 12 patients (12%), consisting of 6 patients with leukemia and 6 with solid organ malignancy. This result supports the available epidemiological data that the percentage of bacteremia patients infected by *E. coli* is associated with particular underlying clinical conditions. A study by Bonten et al. mentioned that the highest rate of patients with bacteremia resulting from *E. coli* was lymphocytic leukemia and multiple myeloma (12–13%). The neoplastic disease has a relative risk (RR) of developing *E. coli* bacteremia 14.9 fold compared with the general population.[13]

The proportion of bacteremia cases with a primary diagnosis of COVID-19 was relatively high, namely 11 patients (26%). Bhatt et al[14] report that the bloodstream infections observed in patients with COVID-19 may have contributed to the more severe presentation and clinical course. Furthermore, it reflects other underlying physiological and immunological complications of COVID-19. Alternatively, a complicated hospital course may have contributed to more risk factors for developing bloodstream infections.[14] In this

research, 26% of COVID patients with co-infection by bloodstream infection due to *E. coli* need attention to the importance of surveillance and prevention of the possibility of a healthcare-associated infection BSIs.

No review of the source of the bloodstream infection was carried out in this study. However, several studies have reported that central line is the most common presumed source of bloodstream infections.[14]

### *Antimicrobial Resistance Profile*

The microorganism was classified based on antimicrobial resistance profiles. Of 43 isolates, 15 (35%) were non-MDRO, 28 (63%) were ESBL-producing microorganisms, 4 (9%) were Carbapenem-resistant. In addition, three ESBL-producing microorganisms were Carbapenem-resistant microorganisms (Table 4).

**Table 4.** Types of Organisms Based on Antimicrobial Resistance Profile

| Types of the Organism | n (%) |
|---|---|
| Non-MDRO | 15 (35) |
| ESBL-producing strain | 24 (56) |
| Carbapenem-resistant strain | 1(2) |
| ESBL-producing strain AND Carbapenem-resistant strain | 3 (7) |
| **Total** | 43 (100) |

The ESBL-producing bacteria were higher than the non-MDRO bacteria, 63% and 35%, respectively. This number was very high. The study showed that the prevalence of ESBL-producing bacteria is increased in the latest period. The clinical relevance of infections caused by ESBL-producing organisms has been outlined in several studies.[15,16] In a retrospective analysis of patients with *E. coli* BSI over four years in a teaching hospital, 58.9% developed ESBL-producing *E. coli*.[17]

No risk factor analysis for ESBL infection in this study, but several studies report that ESBL-producing *E. coli* bacteremia is associated with prior urinary tract infections,[17] previous cephalosporin exposure[17], central venous cathether[15], and history of admission to a long-term care hospital.[18]

Bloodstream infections, particularly BSIs due to MDR *E. coli*, can be caused by hospital-acquired or community-acquired infections. It has been widely reported that infections caused by MDR bacteria are associated with hospital/healthcare-associated infections. Several studies supported community-acquired BSIs by MDR bacteria, which reported the presence of carriers of ESBL-producing *E. coli* bacteria in communities with varying prevalence between different populations. [19,20,21]

Globally, an 8-fold growth in the bowel carriage rate of ESBL *E. coli* in the community during the last decade. The pooled incidence confirmed an upward trend of *E. coli* carriage in the community, growing from 2.6% in 2003–2005 to 21.1% in 2015–2018. Over the entire period, the highest carriage rate happened in South-East Asia (27%), while the lowest happened in Europe (6.0%).[22] In addition, the carrier of ESBL-producing *E. coli* bacteria was reported to develop bloodstream infection.[19]

BSI caused by emerging multidrug-resistant *E. coli* strains is more challenging to treat and confers a higher risk of death. Although it cannot be concluded that the cause of death was purely due to *E. coli* BSI, 68% of patients died in this study. A study reported that in *E. coli* BSI, 50% of the patients died, and the mortality analysis showed that 33.3% of the deaths were associated with BSI.[23]

**Table 5**. Antibiotics Resistant Pattern of *E. coli* Isolated from Bacteremia Hospitalized Patients

| Antibiotics | Tested number | Resistance n (%) |
|---|---|---|
| Ciprofloxacin | 27 | 27 (100) |
| Levofloxacin | 27 | 27 (100) |
| Ampicillin | 42 | 39 (93) |
| Piperacillin | 43 | 32 (74) |
| Tetracyclin | 43 | 31 (72) |
| Cefotaxime | 41 | 27 (66) |
| Cefazolin | 43 | 28 (65) |
| Ceftriaxone | 43 | 28 (65) |
| Trimetoprim-sulfametoxazole | 43 | 28 (65) |
| Aztreonam | 43 | 27 (63) |
| Ceftazidime | 43 | 26 (60) |
| Moxifloxacin | 42 | 25 (58) |
| Cefepime | 37 | 17 (46) |
| Gentamicin | 42 | 15 (36) |
| Ampicillin Sulbactam | 43 | 10 (23) |
| Chloramphenicol | 43 | 8 (19) |
| Amoxicillin-Clavulanate | 42 | 7 (17) |
| Cefoxitin | 37 | 5 (14) |
| Fosfomycin | 42 | 5 (12) |
| Imipenem | 41 | 4 (10) |
| Meropenem | 42 | 4 (10) |
| Tigecycline | 30 | 2 (7) |
| Cefoperazone-Sulbactam | 43 | 2 (5) |
| Amikacin | 43 | 1 (2) |
| Piperacillin Tazobactam | 40 | 0 (0) |

The antimicrobial resistance pattern of tested antimicrobials against *E. coli* from bloodstream infection patients in Table 5 revealed a high proportion of strains of *E. coli* were resistant to Ampicillin (93%), Piperacillin (74%), and tetracycline (72%). The resistance of third-generation cephalosporin ceftriaxone, cefotaxime, and ceftazidime was 66%, 65%, and 60%, respectively, while the fourth-generation cephalosporin cefepime was lower (46%). The resistance to trimethoprim-sulfamethoxazole was 65%. Carbapenem as a drug of choice for multi-drug resistance E coli showed resistance was 10%. A low proportion of strains of *E. coli* were resistant to tigecycline (7%), cefoperazone-sulbactam (5%), and amikacin (2%). No resistance to piperacillin tazobactam was found. Quinolone antibiotics (levofloxacin and ciprofloxacin) were tested only in 27 isolated, and the result was 100% resistance.

The resistance to several drugs, including carbapenem antibiotics (imipenem and meropenem), was meagre. Carbapenems are β lactam antibiotics, as are penicillins and cephalosporins, but differ from these other classes in their exact chemical structure. The bactericidal activity of carbapenem results from the inhibition of cell wall synthesis. Carbapenem penetrates the cell wall of most Gram-positive and Gram-negative bacteria to bind penicillin-binding-protein (PBP) targets.[24] ESBLs are enzymes that inactivate most penicillins, cephalosporins, and aztreonam. ESBL producing bacteria generally remain susceptible to carbapenems. Therefore, it is relevant to the current data that Carbapenem is still an effective drug for treating infections caused by ESBL producers.[25]

High resistance (>60%) to the antibiotics ciprofloxacin, levofloxacin, ampicillin, piperacillin, tetracycline, cefotaxime, cefazolin, ceftriaxone, trimethoprim-sulfamethoxazole, aztreonam, and ceftazidime was shown. This result supports another study that antimicrobial resistance among *E. coli* causing bloodstream infection was common; 36% of *E. coli* blood isolates were non-susceptible to ciprofloxacin, and 23% were non-susceptible to third-generation cephalosporins.[26] ESBLs do not inactivate non–β-lactam agents (eg, ciprofloxacin, trimethoprim-sulfamethoxazole, gentamicin). However, organisms that carry ESBL genes often carry additional genes or mutations in genes that mediate resistance to a broad range of antibiotics.

The number of ESBL-producing *E. coli* is relatively high (63%). ESBLs producing Enterobacteriaceae, including *E. coli,* can hydrolyze broad-spectrum cephalosporins, monobactams, and penicillins. Enzymes of class A β-lactamases, like TEM-1, TEM-2, and SHV-1, are responsible for the resistance to ampicillin, amoxicillin, and early generation cephalosporins. Resistance to third-generation cephalosporins arises when mutation of genes encoding TEM-1, TEM-2,

or SHV-1 gives rise to new β-lactamases that can hydrolyze them.[10]

This study's resistance rate to fluoroquinolones in ESBL-producing E. coli is high (100%). This result supports another study that extended-spectrum β-lactamase (ESBL) constitutes the most common antibiotic resistance mechanism often found on the same resistance plasmids. [27]

These epidemiological data provide good information on the resistance profile of *E. coli* causing BSI in the tertiary referral hospital. The high prevalence of bloodstream infections caused by MDRO E. coli necessitates strict infection control in order to reduce the number of MDRO *E. coli* infections in tertiary hospitals. High levels of antimicrobial resistance encourage clinicians to carry out culture and antibiotic susceptibility testing as soon as possible after the appearance of signs and symptoms of infection to provide definitive and appropriate treatment immediately. While waiting for the definitive antibiotics, the local antibiotic sensitivity pattern in the hospital needs to be taken into account to choose the right empirical antibiotics. Therefore, the role of updated epidemiology data as the guide for empirical antimicrobial therapy is essential.

## CONCLUSIONS

According to epidemiology statistics, 17% of Gram-negative bacteria identified from bloodstream infections were the pathogen *E. coli*. Quinolones, ampicillin, piperacillin, tetracycline, beta-lactam antibiotics, and trimethoprim-sulfamethoxazole were all linked to high levels of antimicrobial resistance. Strict infection control is required due to the high occurrence of bloodstream infections caused by MDRO *E. coli*.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Tropical and Infectious Disease

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This journal is a peer-reviewed journal established to promote the recognition of emerging and reemerging diseases, specifically in Indonesia, South East Asia, other tropical countries and around the world, and to improve the understanding of factors involved in disease emergence, prevention, and elimination.

The journal is intended for scientists, clinicians, and professionals in infectious diseases, biochemistry and molecular biology, microbiology, and related sciences. Established to promote the recognition of emerging and re-emerging diseases, specifically in Indonesia, South East Asia, other tropical countries, and worldwide, and to improve the understanding of factors involved in disease emergence, prevention, and management. We welcome contributions from infectious disease specialists in academics, industry, clinical practice, public health, and pharmacy, as well as from specialists in economics, social sciences, and other disciplines. For information on manuscript categories and the suitability of proposed articles, see below and visit the Guidelines for Authors section.

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Elsevier Saunders; 2012.

2. Calder PC, Field CJ, Gill HS, editors. Nutritional and immune function. Oxon: CABI Publishing; 2002.

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3. Fauci AS, Braunwald E, Kasper DL, Hauser SL, Longo DL, Jameson JL, et al. Harrison's Principles of Internal Medicine. 17th ed. New York: McGraw Hill; 2008.

Chapter in a book

4. Vidyadaran S, Ramasamy R, Seow HF. Stem cells and cancer stem cells: Therapeutic Applications in Disease and Injury. In: Hayat MA, editor. New York: Springer; 2012.

Corporate/Organization as Author

5. Canadian Dental Hygienists Association. Dental hygiene: defi nition and scope. Ottawa: Canadian Dental Hygienists Association; 1995.E-book6. Frank SA. Immunology and Evolution of Infectious Disease [Internet]. Princeton: Princeton University Press; 2002 [cited 2014 December 17]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK2394/pdf/TOC.pdf

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2. Abdullah M, Chai PS, Chong MY, Tohit ERM, Ramasamy R, Pei CP, et al. Gender eff ect on in vitro lymphocyte subset levels of healthy individuals. Cellular Immunology. 2012;272(2):214-9.

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3. Clancy JL, Patel HR, Hussein SM, Tonge PD, Cloonan N, Corso AJ, et al. Small RNA changes enroute to distinct cellular states of induced pluripotency. Nature communications.2014; 5:5522.Epub 2014/12/11.

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- Results
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- Conclusions
- Acknowledgements
- Conflict of Interest
- References (minimum 25 references)

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