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**Research Article**

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# Antimicrobial Resistance in *Salmonella enterica* serovar Typhi mediated by efflux pump in Makassar Sulawesi Selatan

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**ABSTRACT**

*Antibiotic resistance is a global public health threat, but nowhere is it as stark as in Indonesia. Research related to antimicrobial resistance and its mechanism to the existing health care situation in each country is a big challenge. This research explains about antimicrobial resistance in Salmonella enterica serovar Typhi mediated by efflux pump in Makassar Sulawesi Selatan using disk diffusion method which added efflux pump inhibitor (CCCP) 100 µM. From 20 selected patients, we found 14 isolates with characteristic: 11 isolates are MDR and 5 isolates sensitive to drug and effected to CCCP. Eight isolates resistant to chloramphenicol; 11 isolates are resistant to cotrimoxazole; 3 isolates each are resistant to tetracycline, amoxicillin and ciprofloxacin. In conclusion, while antimicrobial resistance was mediated by efflux pump in Makassar, multiple resistance was most often acquired.*

**Keywords***: Resistance, Salmonella Typhi,* CCCP, efflux pump.

## INTRODUCTION

Typhoid fever remains an important public health problem in developing countries caused Salmonella. Approximately, over 2.16 million episodes of typhoid occurred worldwide and more than 90% of this morbidity and mortality occurred in Asia [1]. Typhoid fever is still a major health problem in Indonesia and the prevalence in South Sulawesi [2]. One of the risk factors difficult to eradicate this disease is easily mutated and transmitted [3].

Uncontrolled use of antibiotics leads to high incidence of multidrug-resistant (MDR), especially for first-line antimicrobials, such as ampicillin, chloramphenicol, and cotrimoxazole [4]. There are two mechanisms of drug resistance in *S.* Typhi: plasmid-mediated and chromosomal DNA-mediated mechanism. [5]. However, other mechanisms such as a decrease in permeability and active efflux antimicrobials may also be involved. Resistance caused by overexpression of the bacterial efflux pump has not been studied mutations in the target genes of antibiotics or the formation of enzymes that can deactivate antibiotics [6].

Efflux pumps are through transport proteins able to carry out the toxic substrates (including antibiotics) from within cell into the external environment. These proteins are found in both Gram-positive and negative bacteria as well as in eukaryotic organisms. Ten families have identified as antibiotic efflux pump, distributed in five super-families: small multidrug resistance (SMR), multidrug endosomal resistance (MET) and major facilitator superfamily (MAR) [7].

This is the first study in Makassar-Indonesia that identifies resistance mediated by efflux pump in *Salmonella enterica serovar* Typhi isolates.

## EXPERIMENTAL SECTION

### Place and time

The research was conducted during January 2011 to April 2011. Bacterials isolates derived from hospitals and Public Health Centers in Makassar, South Sulawesi-Indonesia.

### Disk diffusion test

The disk diffusion susceptibility method is simple and practical and has been well-standardized with modification [8]. The test is performed by applying isolated *S. typhi* inoculum of approximately $1\times10^8$ CFU/mL to the surface of Mueller-Hinton agar plate. Paper antibiotic disks containing: amoxicillin (25µg), tetracycline (30µg), sulfamethoxazole-trimethoprim (75µg), chloramphenicol (30µg), and Ciprofloxacin (5µg) were placed on the inoculated agar surface. The diameters of the zone inhibitor of each drug interpreted using the criteria published by the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards or NCCLS) [9].

### EP inhibition assay

The EP inhibition assay used disk diffusion test as descried previously [8, 10] with modification, by adding efflux pump inhibitor CCCP (Sigma) 100 µM and inoculated for 18-24 hours.

## RESULTS AND DISCUSSION

Twenty isolates of *S.* Typhi obtained from the blood of typhoid fever patients comprised of MDR isolates: 3 resistant to 1 antibiotic and 6 isolates are sensitive to all drugs. There were 11 isolates resistant to cotrimoxazole, 8 to chloramphenicol, and 3 isolates each were resistant to tetracycline, amoxicillin and ciprofloxacin (Table 1).

**Table 1. Characterization of isolated *S.* typhi to antimicrobials**

| Isolate | Zone of Inhibition (mm) and the rate of antimicrobial sensitivity | | | | | | | | | | Note | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Chloramphenicol | | Amoxicillin | | Tetracycline | | Cotrimoxazole | | Ciprofloxacin | | | | |
| 1 | 12 | R | 24 | S | 10 | R | 8 | R | 34 | S | MDR | | |
| 2 | 16 | R | 22 | S | 19 | S | 8 | R | 33 | S | MDR | | |
| 3 | 15 | R | 8 | R | 15 | S | 24 | S | 27 | S | MDR | | |
| 4 | 11 | R | 20 | S | 12 | I | 8 | R | 30 | S | MDR | | |
| 5 | 14 | R | 8 | R | 14 | I | 21 | S | 29 | S | MDR | | |
| 6 | 18 | S | 23 | S | 21 | S | 10 | R | 17 | R | MDR | | |
| 7 | 22 | S | 21 | S | 19 | S | 8 | R | 18 | R | MDR | | |
| 8 | 25 | S | 22 | S | 27 | S | 8 | R | 33 | S | | R | |
| 9 | 31 | S | 29 | S | 26 | S | 28 | S | 33 | S | | | S |
| 10 | 21 | S | 31 | S | 29 | S | 22 | S | 32 | S | | | S |
| 11 | 10 | R | 29 | S | 26 | S | 12 | R | 22 | S | MDR | | |
| 12 | 8 | R | 31 | S | 30 | S | 18 | S | 21 | I | MDR | | |
| 13 | 25 | S | 30 | S | 29 | S | 12 | R | 34 | S | | R | |
| 14 | 29 | S | 30 | S | 33 | S | 24 | S | 36 | S | | | S |
| 15 | 25 | S | 32 | S | 30 | S | 8 | R | 34 | S | | R | |
| 16 | 31 | S | 31 | S | 26 | S | 27 | S | 33 | S | | | S |
| 17 | 30 | S | 22 | S | 29 | S | 30 | S | 35 | S | | | S |
| 18 | 30 | S | 26 | S | 27 | S | 28 | S | 36 | S | | | S |
| 19 | 30 | S | 8 | R | 30 | S | 8 | R | 34 | S | MDR | | |
| 20 | 10 | R | 31 | S | 31 | S | 8 | R | 29 | S | MDR | | |
| Total | | 8 | | 3 | | 3 | | 11 | | 3 | 11 | 3 | 6 |

*Note: R= resistance, S= sensitive, MDR= multi drug resistance*

Among Isolated of *S.* Typhi resistant which resistance was mediated by efflux pump (affected by carbonyl cyanide-m-chlorophenyl-hydrazone CCCP), 6 isolates suffered large increase inhibition zone while 2 isolates showed relatively less (Table 2).

**Table 2. Characteristics of isolated *S.* Typhi resistant mediated by efflux pump**

| Isolate | Zone of inhibition (mm) before and after treaded with CCCP | | | | | | | | | | Note |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Chloramphenicol | | Amoxicillin | | Tetracycline | | Cotrimoxazole | | Ciprofloxacin | | |
| | A | B | A | B | A | B | A | B | A | B | |
| 1 | 12 | 13* | 24 | 20 | 10 | 9 | 8 | 8 | 34 | 36* | Efflux |
| | R | R | S | S | R | R | R | R | S | S | (↑ small) |
| 2 | 16 | 16 | 22 | 18 | 19 | 9 | 8 | 8 | 33 | 33 | |
| | R | R | S | S | S | R | R | R | S | S | |
| 3 | 15 | 11 | 8 | 8 | 15 | 9 | 24 | 8 | 27 | 25 | |
| | R | R | R | R | S | R | S | R | S | S | |
| 4 | 11 | 16* | 20 | 8 | 12 | 14* | 8 | 24* | 30 | 24 | Efflux |
| | R | R | S | R | I | I | R | S | S | S | (↑ large) |
| 5 | 14 | 25* | 8 | 10* | 14 | 9 | 21 | 8 | 29 | 19 | Efflux |
| | R | S | R | R | I | R | S | R | S | I | (↑ large) |
| 6 | 18 | 11 | 23 | 10 | 21 | 11 | 10 | 8 | 17 | 19* | Efflux |
| | S | R | S | R | S | R | R | R | R | I | (↑ small) |
| 7 | 22 | 16 | 21 | 9 | 19 | 9 | 8 | 8 | 18 | 12 | |
| | S | R | S | R | S | R | R | R | R | R | |
| 8 | 25 | 24 | 22 | 21 | 27 | 24 | 8 | 8 | 33 | 33 | |
| | S | S | S | S | S | S | R | R | S | S | |
| 9 | 31 | 26 | 29 | 28 | 26 | 27 | 28 | 10 | 33 | 32 | |
| | S | S | S | S | S | S | S | R | S | S | |
| 10 | 21 | 20 | 31 | 31 | 29 | 28 | 22 | 22 | 32 | 31 | |
| | S | S | S | S | S | S | S | S | S | S | |
| 11 | 10 | 8 | 29 | 27 | 26 | 24 | 12 | 8 | 22 | 32 | |
| | R | R | S | S | S | S | R | R | S | S | |
| 12 | 8 | 14* | 31 | 16 | 30 | 14 | 18 | 9 | 21 | 20 | Efflux |
| | R | R | S | S | S | I | S | R | I | I | (↑ large) |
| 13 | 25 | 15 | 30 | 19 | 29 | 13 | 12 | 25* | 34 | 35* | Efflux |
| | S | R | S | S | S | I | R | S | S | S | (↑ large) |
| 14 | 29 | 29 | 30 | 29 | 33 | 34 | 24 | 23 | 36 | 35 | |
| | S | S | S | S | S | S | S | S | S | S | |
| 15 | 25 | 26 | 32 | 31 | 30 | 30 | 8 | 8 | 34 | 34 | |
| | S | S | S | S | S | S | R | R | S | S | |
| 16 | 31 | 30 | 31 | 30 | 26 | 25 | 27 | 21 | 33 | 33 | |
| | S | S | S | S | S | S | S | S | S | S | |
| 17 | 30 | 31 | 22 | 22 | 29 | 28 | 30 | 20 | 35 | 34 | |
| | S | S | S | S | S | S | S | S | S | S | |
| 18 | 30 | 30 | 26 | 25 | 27 | 28 | 28 | 19 | 36 | 31 | |
| | S | S | S | S | S | S | S | S | S | S | |
| 19 | 30 | 28 | 8 | 31* | 30 | 26 | 8 | 8 | 34 | 34 | Efflux |
| | S | S | R | S | S | S | R | R | S | S | (↑ large) |
| 20 | 10 | 29* | 31 | 23 | 31 | 28 | 8 | 8 | 29 | 31* | Efflux |
| | R | S | S | S | S | S | R | R | S | S | (↑ large) |

*Note: (A) before treated by CCCP; (B) after treated by CCCP; (*) Zone of inhibition after added of CCCP; (↑) Increase inhibition zone*

Antimicrobial resistance is an important concern for the public health authorities at global level, it has become the the major public health problems especially in developing countries due to higher incidence of inappropriate use of antibiotics [11]. In Indonesia the infectious disease burden is among the highest in the world and recent report showed the inappropriate and irrational use of antimicrobial agents against these diseases [12]. Resistance caused by overexpression of the pump efflux bacteria has not been studied in comparison with mutations in the target genes of antibiotics or the formation of enzymes that can deactivate antibiotics [6].

From 20 selected patients, we found 14 isolates with characteristic: 11 isolates are MDR and 5 isolates sensitive to drug and effected to CCCP. Eight isolates resistant to chloramphenicol; 11 isolates are resistant to cotrimoxazole; 3 isolates are each resistant to tetracycline, amoxicillin and ciprofloxaine, respectivelly. Particularly troubling because the resistance to ciprofloxacin was also found, this drug is the drug recommended by WHO in the event of MDR *S. typhi* [13].

Increased resistance toin the event of drugs such as chloramphenicol, trimethoprim, ampicillin, streptomycin, tetracycline, and several other groups have led to the use of fluoroquinolone to resolve infections. However, bacteria resistant to ampicillin, chloramphenicol, streptomycin, and cotrimoxazole were known able to degrade the membrane permeability or increase the efflux pumping action thus helping to accelerate the declined sensitivity to ciprofloxacin [14].

The bacterial resistance to antibiotics is reported as a major cause of health problems in the world, and the MDR bacteria over expression efflux pump result in reduced effectiveness by among reducing the intracellular concentration of the antibiotic [15]. Efflux pump is major mechanism of bacterial acting resistance to antibiotics and it's broad distribution causes a lack of intrinsic vulnerability and the occurrence of cross-resistance to many different drug classes [7, 16].

Efflux pumps are found in almost all bacterial species. The genes encoding this class of proteins can be located on chromosomes or plasmids [17, 18]. efflux pump in *S. Typhi* is of the RND family we proved in this reserach the inhibition of rflux pump in 6 isolates of *S. Typhi*.

According to their composition, number of transmembrane spanning regions, energy sources and substrates, bacterial efflux pumps are classified into five families: the resistance-nodulation-division (RND) family, the major facilitator superfamily (MFS), the ATP (adenosine triphosphate)-binding cassette (ABC) superfamily, the small multidrug resistance (SMR) family [a member of the much larger drug/metabolite transporter (DMT) superfamily], and the multidrug and toxic compound extrusion (MATE) family [17-19].

## CONCLUSION

To antimicrobial through efflux pump mechanism was detected in Makassar isolates of *S. Typhi*.

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