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Antibiogram and biofilm formation among extended-spectrum β -lactamase-producing *Klebsiella pneumoniae* clinical isolates in Sanglah General Hospital, Bali, Indonesia



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ABSTRACT

Background: *Klebsiella pneumoniae* is a common cause of healthcare-associated infections (HAIs), and has a high level of resistance to antibiotics first, second, extended-spectrum cephalosporins, and monobactam which are a serious threat to public health worldwide. Besides, it is known that this bacterium can form biofilms as virulence factors that contribute to drug resistance. This study aims to determine the antibiotics susceptibility patterns and the capacity of *K. pneumoniae* to form biofilms.

Methods: *K. pneumoniae* was isolated from clinical specimens (urine, sputum, pus, blood, and others) for the period 2018-2019. Bacterial identification and antibiotic susceptibility testing were performed using the Vitek Compact 2 (bioMérieux[®]) test in the Clinical Microbiology Laboratory of Sanglah General Hospital. Biofilm formation was checked using the tissue culture plate method (TCP). Data were analyzed using SPSS

version 20 for Windows.

Results: Most of Extended Spectrum β -Lactamase (ESBL)-producing *K. pneumoniae* showed resistance to antibiotics. The susceptible profiles were only towards ertapenem (97.50%), meropenem (97.50%), amikacin (95.00%), and tigecycline (87.50%). The TCP method detected 72 (90.00%) as biofilm producers among 80 clinical isolates, while 8 (10.0%) as non-biofilm producers. Among the biofilm-producer bacteria, there were 6 (7.50%) as strong, 37 (46.25%) moderate, and 29 (36.25%) weak biofilm-producer isolates.

Conclusions: Most ESBL-producing *K. pneumoniae* clinical isolates in Sanglah General Hospital demonstrate multiple resistance to antibiotics and as biofilm producers. However, further research is needed to be conducted using a molecular approach to see the ESBL- and biofilm-encoded genes.

Keywords: *Klebsiella pneumoniae*, Multidrug Resistance, Antibiogram, Tissue Culture Plate, Crystal Violet, Biofilm.

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INTRODUCTION

Healthcare-associated infections (HAIs) are infections that occur in patients during treatment at a health care facility and are associated with high morbidity and mortality.¹ The ESKAPE bacterial group consisting of *E. faecium*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *Enterobacter spp* has been established as HAIs pathogens.²

K. pneumoniae, as one of the ESKAPE pathogens, is a normal flora in the human body that colonizes the gastrointestinal

tract belonging to the *Enterobacteriaceae* family.^{2,3} This bacterium causes bacteremia, septicemia, urinary tract infections, surgical site infections, pneumonia, and meningitis in neonates and premature infants in the hospital.^{3,4} In certain situations, *K. pneumoniae* can produce extended-spectrum β -lactamases (ESBLs), which show resistance to first, second, extended-spectrum cephalosporins, and monobactam.⁵⁻⁷ Moreover, this bacterium has essential virulence factors such as biofilms. Biofilm is a collection of microbial cells attached irreversibly to a bacterial

surface and encased in the Extracellular Polymeric Substances (EPS) matrix.⁸ This matrix is composed of protein (<1-2%), DNA (<1%), RNA (<1%), polysaccharides (1-2%), and water (>97%).⁸ In the biofilm, bacteria produce chemotactic particles to communicate with other bacteria; this is called quorum sensing.⁹ The ability to produce biofilm causes a slowdown in the penetration of antibiotics into the biofilm layer and causes prolonged treatment.^{8,9}

One of the detection methods to evaluate biofilm production capacity is the TCP method. TCP is the gold-

standard quantitative testing method for biofilm formation.¹⁰ Antibiofilm formation of ESBL-producing *K.pneumoniae* is important to address the growing problem of antimicrobial resistance worldwide. However, data on antibiotics susceptibility pattern and biofilm production capacity ESBL-producing *K.pneumoniae* are very limited in Indonesia, especially in Bali. Based on those mentioned above, this study aims to determine the antibiotics susceptibility pattern and biofilm production of ESBL-producing *K.pneumoniae* isolated from clinical isolates at Sanglah General Hospital, Bali, Indonesia.

METHODS

ESBL-producing *K.pneumoniae* isolates from clinical samples that were glycerol-stocked in the Clinical Microbiology Laboratory of Sanglah General Hospital, Denpasar, for the 2018-2019 period, were used in this study. The identification and antibiotics susceptibility test assayed using the Vitek Compact 2 (bioMérieux®) based on the manufacturer's instruction.

Biofilm formation assayed using the TCP method described by Christensen GD et al., with slight modification.¹¹ Bacterial cultures were inoculated in trypticase soy broth (TSB) with 1% glucose. After that, incubate the broths overnight at 37°C. Individual wells Corning® 96-well clear flat bottom polystyrene microplate were inserted with 200 µl of the bacterial suspension. The negative control (TSB with 1% glucose) was also incubated and added to the microplate. The microplates were sealed and incubated at 37° C for 48 hours. Then, the microplates were inverted and washed with 0.2 mL of phosphate buffer saline twice. Furthermore, the microplates were fixed by giving 2% sodium acetate and stained by adding 200 µl of 0.1% crystal violet. The excess stain was removed by carefully using distilled water and dried at room temperature. After the microplate was dry, add 200 µl of 96% ethanol to the microplate and inverted quickly. Finally, do the reading using a microplate reader at 620nm. The test was carried out in triplicate and repeated three times based on a previous study.¹² The reading result is an absorbance or Optical Density (OD), which describes the quantity of biofilm

Table 1. Distribution of biofilm production by ESBL-producing *K.pneumoniae* based on specimen type

Specimen type	Isolates (N=80)
Urine, n (%)	27 (33.75)
Sputum, n (%)	25 (31.25)
Pus, n (%)	12 (15.00)
Blood, n (%)	11 (13.75)
Others, n (%)	5 (6.25)

Table 2. Distribution of ESBL-Kp based on category of biofilm production

Category of Biofilm Producer	Isolates (N=80)
Non-producer, n (%)	8 (10.00)
Weak producer, n (%)	29 (36.30)
Moderate producer, n (%)	37 (46.30)
Strong producer, n (%)	6 (37.50)

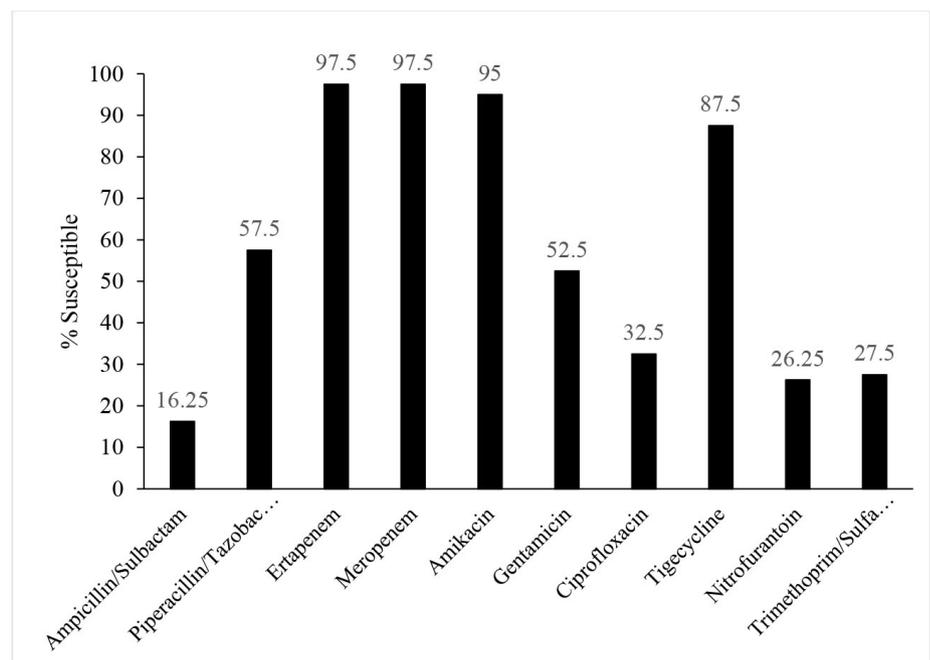


Figure 1. Antibiofilm of ESBL-Kp isolated in Sanglah General Hospital in 2018-2019

formation adjusted to Stepanović's criteria such as: 1) None ($OD < ODc$); 2) Weak ($ODc < OD \leq 2 \times ODc$); 3) Moderate ($2 \times ODc < OD \leq 4 \times ODc$); and 4) Strong ($OD > 4 \times ODc$). ODc is optical density cut-off calculated by average OD of negative control plus three times standard deviation (SD) of the negative control.¹³ The data in this study were analyzed with IBM SPSS® Statistics 20.0 software. The variables were described to frequencies and percentages.

RESULTS

There were 80 isolates obtained from clinical specimen stocks (urine, sputum,

pus, blood, others.) at the Clinical Microbiology Laboratory of Sanglah Hospital, Denpasar, for the period 2018-2019 (Table 1). Based on the specimen distribution of biofilm production by ESBL-producing *K.pneumoniae*, it was predominant by urine (33.75%), followed by sputum (31.25%), pus (15.00%), blood (13.75%), and others (6.25%) (Table 1).

Antibiotics susceptibility pattern of ESBL-producing *K.pneumoniae* in this study showed that 97.50% of the isolates were still susceptible to ertapenem and meropenem, 95.00% to amikacin, and 87.50% to tigecycline (Figure 1). On the

other hand, most isolates showed resistance to ampicillin/sulbactam, piperacillin/tazobactam, gentamicin, ciprofloxacin, nitrofurantoin, and trimethoprim/sulfamethoxazole (Figure 1).

From the 80 clinical isolates, the TCP method detected 72 (90.00%) as biofilm producers and 8 (10.00%) as non-biofilm producers. Among the biofilm-producer bacteria, there were 6 (7.50%) as strong, 37 (46.25%) moderate, and 29 (36.25%) weak biofilm-producer isolates (Table 2).

DISCUSSION

K. pneumoniae has been recognized as HAIs pathogens due to the emergence of ESBL producing and biofilm-forming as a major health problem worldwide.^{2,14} This bacterium is one of the important causes of multidrug-resistance infections with increased mortality and hospital stay duration, resulting in increased healthcare costs and responsible for many complicated bacterial infections to treat, such as cystic fibrosis, chronic wounds, and chronic media otitis.^{15,16}

In the present study, we found that most of *K. pneumoniae* was resistant to broad range antibiotics, with ampicillin/sulbactam, piperacillin/tazobactam, gentamicin, ciprofloxacin, nitrofurantoin, and trimethoprim/sulfamethoxazole, while ertapenem, meropenem, amikacin, and tigecycline, showing 97.50%; 97.50%; 95.00%; 87.50% had good susceptibility, respectively. This finding is suchlike to Nirwati H et al., who reported that *K. pneumoniae* had good susceptibility to meropenem (98.60%), amikacin (95.80%), piperacillin /tazobactam (90.00%).¹⁷ Besides, Lin WP et al., reported that *K. pneumoniae* had good susceptibility to ertapenem (97.60%), meropenem (99.30%), amikacin (94.70%), tigecycline (97.80%), and piperacillin/tazobactam (91.30%).¹⁸

There are various methods for detecting biofilms, including tissue culture plate (TCP), tube method (TM), congo red agar method (CRA). In this study, a biofilm formation test was carried out on 80 isolates using the tissue culture plate method. Based on Asati S and Chaudhary U study, the TCP method is more straightforward, more sensitive, and helps

in the quantitative assessment of biofilm formation compared to the TM and CRA method.¹⁹

Based on the TCP method, we found 72 (90.00%) as biofilm producers and 8 (10.00%) as non-biofilm producers. This result is similar to the previous study by Seifi K et al., who found that out of 94 *K. pneumoniae* samples tested, 88 (93.60%) of the isolates generated biofilm.³ Some studies, such as Alcántar-Curiel MD et al., reported that the majority of *K. pneumoniae* 69.00% as strong, 20.30% as weak to produced biofilm, and 10.10% were non-biofilm producers.²⁰ Hassan A et al., reported that 70 (64.70%) as high/medium, and 40 (35.30%) as weak biofilm producers.¹²

Several factors cause the difference capacity of biofilm formation to affect biofilm formation, including the physicochemical properties of *K. pneumoniae*, physical interactions between its constituents, the type of surface on which the biofilm is attached, the ability of biofilm adhesion, temperature, pH, and nutrition.²¹ In this study, 1% glucose was added to the TSB medium to help form biofilms. Similar to Hassan A et al., adding 1% glucose in TSB medium to induce biofilm formation in *S. epidermidis*, *E. coli*, *K. pneumoniae*, *S. aureus*, *E. faecalis*, and *P. aeruginosa*.¹² In another study, Asati S and Chaudhary U also used the TSB with 1% glucose to induce biofilm formation in *Klebsiella* spp., *E. coli*, *Citrobacter* spp., *Proteus* spp., and *Enterobacter* spp.¹⁹

CONCLUSIONS

Most ESBL producing-*K. pneumoniae* clinical isolates in Sanglah General Hospital, Denpasar, have good susceptibility to carbapenem, amikacin, and tigecycline, indicating that these antibiotics can be considered empirical therapy for ESBL-producing bacterial infection. Almost all isolates are biofilm producers, suggesting that biofilm production capacity is an essential virulence factor for this bacterium-however, molecular characterization to detect ESBL producing-*K. pneumoniae* and biofilm production-encoded genes are necessary to be conducted.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

Research Ethics Committee, Faculty of Medicine, Udayana University, Denpasar, Bali, Indonesia, permitted this study with number: 473/UN.14.2.2.VII.14/LP/2020.

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AUTHOR CONTRIBUTIONS

All authors equally contribute to the study from the conceptual framework, literature search, data acquisition, data analysis, manuscript preparation until reporting the study results through publication.

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