

Susceptibility of *Klebsiella Pneumoniae* Isolated from Pus Specimens of Post-Surgery Patients in Medan, Indonesia to Selected Antibiotics

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Abstract

AIM: This study was to determine the sensitivity of *Klebsiella pneumoniae* isolated from pus specimens of post-surgery patients in Medan, Indonesia to selected antibiotics.

METHODS: Samples were collected at the Laboratory of Microbiology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia. The isolated bacteria were identified by Gram's stain, colony characteristics, and biochemical tests. Susceptibility of *K. pneumoniae* isolates were tested to selected antibiotics including amikacin, meropenem, levofloxacin, ciprofloxacin, co-trimoxazole, ceftazidime, cefoperazone, cefuroxime, cefepime, cefotaxime, tetracycline, chloramphenicol, amoxicillin and ampicillin with Kirby Bauer method by measuring the inhibitory zone.

RESULTS: A total of 20 *K. pneumoniae* isolates were obtained in this study. The results showed that *K. Pneumoniae* isolates exhibited good sensitivity to amikacin (100%) and meropenem (80%). Sensitivity of levofloxacin (60%), ceftazidime (55%), ciprofloxacin (55%), cefoperazone (50%), and co-trimoxazole (50%) were moderate for the bacterial isolates. *K. Pneumoniae* isolates indicated low sensitivity to cefuroxime (45%), chloramphenicol (35%), cefepime (30%), cefotaxime (30%), tetracycline (30%), amoxicillin (5%), and ampicillin (5%).

CONCLUSION: This study concludes that *K. pneumoniae* isolates are most sensitive to amikacin and less sensitive to ampicillin and amoxicillin.

Introduction

Klebsiella pneumoniae belongs to the family of *Enterobacteriaceae*, it is a Gram-negative bacteria, non-motile, and aerobic rod-shaped bacteria. Their mucoid colonies grow on agar media and are capable of fermenting lactose [1]. *K. pneumoniae* commonly presents in sewage, surface water, soil, and plants, as well as on mucosal surface of mammals. In human, this bacteria is found as saprophyte in nasopharyngeal and intestinal tracts. The bacteria cause hospital-acquired infections including respiratory tract infections, urinary tract infections, and bloodstream infections [2]. In addition, *K. pneumoniae* is also identified on wound after skin surgery [3].

World Health Organization reported resistance of *K. pneumoniae* to third-generation cephalosporins and carbapenems in the world [4]. This situation causes treatment of infectious diseases become difficult and produce more serious problem for the human life [5]. The emergence of resistant *K. pneumoniae* strain to antibiotics in some Asian countries has also been reported [6], [7], [8], [9], [10]. However, there are limited informations regarding the susceptibility of the bacteria to antibiotics in Indonesia regions. Hence this study was performed to investigate the susceptibility of *K. Pneumoniae* isolated from pus specimens of post-surgery patients in Medan, Indonesia.

Material and Methods

Chemicals

Bacterial growth media including brain heart infusion agar, Mac Conkey agar, eosin methyl blue agar, sugarsbroth, triple sugar iron agar, urea broth, methyl red media, Voges-Proskauer media, and Mueller Hinton agar (MHA) were obtained from Oxoid (Hampshire, UK). Paper discs containing standard antibiotics namely ampicillin 10 µg, amoxicillin 25 µg, chloramphenicol 30 µg, cefuroxime 30 µg, cefotaxime 30 µg, cefoperazone 75 µg, cefepime 30 µg, meropenem 10 µg, amikacin 30 µg, tetracycline 30 µg, ciprofloxacin 5 µg, levofloxacin 5 µg, co-trimoxazole 25 µg, and ceftazidime 30 µg were purchased from Oxoid (Hampshire, UK). Reagents (crystal violet, 96% ethanol, iodine, safranin O, ammonium oxalate, oksalat, para-dimethylaminobenzaldehyde, butanol, acid chloride, α-naphthol 5%, KOH 40%, and distilled water) were supplied by Microbiology Laboratory, Faculty of Medicine, Universitas Sumatera Utara (Medan, Indonesia).

Sample collection

Pus specimens of post-surgery patients in Medan, Indonesia were collected in the Microbiology Laboratory, Faculty of Medicine, Universitas Sumatera Utara from August 2, to September 7, 2016.

Antibiotic susceptibility testing of bacterial isolates

The specimens were aseptically transferred into brain heart infusion, cultured on eosin methyl blue agar plates, and then incubated overnight at 37°C. After 24 hours, isolated colony was identified by observing their characteristics through Gram's staining, viable colonies, motility test, and biochemical tests such as indole, methyl red, Voges-Proskauer, Simon's citrate, urease, and sugars fermentation [11]. A Kirby-Bauer disc diffusion method from the Clinical and Laboratory Standard Institute (2016) was adopted to investigate the antibiotic susceptibility of *K. pneumoniae* isolates [12].

Results

Bacterial isolates characteristics

The results indicated that the bacterial colonies were mucoid, large dome shaped and pink in colour on eosine methylene blue agar media (Figure 1A). The bacterial isolates were rod-shaped and pink

colour with Gram staining which indicated Gram-negative bacteria (Figure 1B). Biochemical testing of the bacterial isolates produced positive results with Voges-Proskauer, Simmons' citrate, and sugar fermentation tests, but negative reactions were identified by Indol, methyl red, and motility tests. The similar results have also been reported by Patel et al., (2017) and Abdullah and Zghair (2016) [13], [14]. Accordingly, these bacterial isolates characteristics were specific for *K. pneumoniae*. A total of 20 *K. pneumoniae* isolates were obtained in this study.

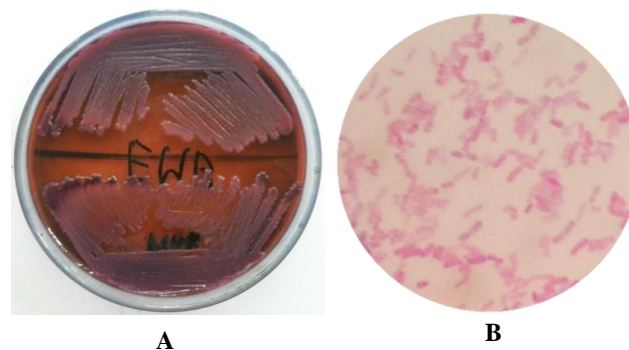


Figure 1: Characteristics of *Klebsiella pneumoniae* on eosin methylene blue agar media (A) and Gram staining (B)

Sensitivity patterns of *K. pneumoniae* to selected antibiotics

In the present study, susceptibility testing of *K. pneumoniae* isolates from pus specimens of post-surgery patients to several antibiotics was determined by measuring the bacterial growth inhibition zone around the antibiotic discs. The bacterial susceptibility to antibiotics is classified into three criteria, namely sensitive, intermediate, and resistant [12]. The results demonstrated that *K. Pneumoniae* isolates produced different sensitivity to antibiotics class (Table 1).

Table 1: Sensitivity patterns of *K. pneumoniae* isolated from pus specimens of post-surgery patients to selected antibiotics

Antibiotic Class	Antibiotic's Name	Isolate Number (%)		
		S	I	R
Aminoglycoside	Amikacin	20 (100.0%)	0 (0.0%)	0 (0.0%)
Carbapenem	Meropenem	16 (80.0%)	1 (5.0%)	3 (15.0%)
	Levofloxacin	12 (60.0%)	1 (5.0%)	7 (35.0%)
Fluoroquinolone	Ciprofloxacin	11 (55.0%)	1 (5.0%)	8 (40.0%)
	Co-trimoxazole	10 (50.0%)	0 (0.0%)	10 (50.0%)
Drug Combination	Ceftazidime	11 (55.0%)	2 (10.0%)	7 (35.0%)
	Cefoperazone	10 (50.0%)	5 (25.0%)	5 (25.0%)
	Cefuroxime	9 (45.0%)	2 (10.0%)	9 (45.0%)
	Cefepime	7 (35.0%)	7 (35.0%)	6 (30.0%)
Cephalosporin	Cefotaxime	6 (30.0%)	1 (5.0%)	13 (65.0%)
	Chloramphenicol	7 (35.0%)	0 (0.0%)	13 (65.0%)
Tetracycline	Tetracycline	6 (30.0%)	2 (10.0%)	12 (60.0%)
Penicillin	Ampicillin	1 (5.0%)	0 (0.0%)	19 (95.0%)
	Amoxicillin	1 (5.0%)	0 (0.0%)	19 (95.0%)

Discussion

Although some bacterial isolates exhibited good sensitivity, but the emergence of bacterial resistance to antibiotics tested also detected.

Ampicillin and amoxicillin which are classified into penicilline derivatives were relatively inactive to *K. pneumoniae* with the number of resistant isolates of more than 90%. According to Ravichitra et al., (2014), *K. pneumoniae* isolated from pus, sputum, and urine samples also resistant to some antibiotics, especially amoxycylav and ofloxacin [15]. Penicillin resistance is due to the ability of *K. pneumoniae* to carry plasmids producing beta-lactamase variants [16]. As we know that beta-lactamase production is the most common mechanism among Gram negative bacteria [17].

This study also indicated that chloramphenicol and tetracycline have low sensitivity to *K. pneumoniae* isolates. The similar result has been reported by other researchers [18], [19]. Chloramphenicol resistance is commonly caused by enzymes activity which add acetyl groups to antibiotics. Acetylated chloramphenicol cannot be bound to the 50S subunit of the bacterial ribosome, so it is unable to inhibit protein synthesis. In addition, the bacteria resistant carries a plasmid with a gene that codes for chloramphenicol acetyltransferase. This enzyme inactivates chloramphenicol pass through the plasma membrane and enters the cell [20]. The low sensitivity of tetracycline in *K. Pneumonia* due to the mutations in the chromosomes in the outer membrane of bacteria that, it leads to the decreasing of tetracyclines penetration into the cell [21].

Moderate sensitivity of co-trimoxazole, cephalosporin, and fluoroquinolone in *K. pneumoniae* isolates has been detected in our study. This finding also supported by other researchers [22], [23], [24]. Co-trimoxazole, a combination of trimethoprim and sulfamethoxazole, blocks the folate synthesis pathway in bacteria. Sulfamethoxazole inhibits the enzyme responsible for the incorporation of para-aminobenzoic acid (PABA) into a precursor of folic acid, therefore blocking folic acid production in bacteria. Trimetoprim is a potent inhibitor of the enzyme dihydrofolatereductase and interferes with the conversion of folic acid to folinic acid. Folinic acid is required in the production of purine as the backbone of the bacterial DNA. Co-trimoxazole resistance is due to the bacterial capability to produce an enzyme as alternative target which resistant to antibiotic inhibition [25]. *K. pneumoniae* can also develop biofilm-forming mechanism to survive under prolonged exposure of antibiotics such as ciprofloxacin [26], gentamicin, and cefotaxime [27]. The change of the target and decrease the accumulation of fluoroquinolones caused by the impermeability of the membrane and excessive expression of the efflux pump mechanisms of this bacteria resistant to fluoroquinolones [28]. Fluoroquinolone class of antibiotic resistance caused by mutations in the gene encoding the DNA gyrase enzyme produced active cause but cannot be bound by fluoroquinolone [29].

In addition, resistance of *K. pneumoniae* isolated from pus specimens to carbapenem class were also detected in this study. Carbapenems are

highly stable to beta-lactamase hydrolysis, so it is a drug of choice for treatment of serious infections caused by *K. pneumoniae* producing extended spectrum beta-lactamase [30]. However, the bacterial resistance to carbapenem is possible since *K. pneumoniae* capable to produce an enzyme which called carbapenemase [31].

Interestingly, all of *K. pneumoniae* isolates were sensitive to amikacin. A study by Simanjuntak (2014) also found that the bacteria isolated from urine of patients with infected urinary tract [19]. Amikacin is an aminoglycoside antibiotic that inhibits protein synthesis in bacteria. This antibiotic binds to the 30S ribosomal subunit mRNA cause reading errors, so bacteria cannot synthesize proteins for growth. Amikacin is also highly resistant to modification by the bacterial enzymes leading many bacteria are sensitive to this antibiotic [32].

In conclusion, *K. pneumoniae* isolated from pus specimens of post-surgery patients in Medan, Indonesia has been resistant to ampicillin, amoxicillin, cefepim, cefotaxime, cefuroxyme, cefoperazone, ceftazidime, tetracycline, chloramphenicol, co-trimoxazole, ciprofloxacin, levofloxacin, and meropenem. However, the bacterial has shown good sensitivity to amikacin.

References

- Martin RM, Bachman MA. Colonization, infection, and the accessory genome of *Klebsiella pneumoniae*. *Front Cell Infect Microbiol*. 2018; 8(4). <https://doi.org/10.3389/fcimb.2018.00004> PMID:29404282 PMCID:PMC5786545
- Podschun R, Ullmann U. *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin Microbiol Rev*. 1998; 11(4):589-603. <https://doi.org/10.1128/CMR.11.4.589> PMID:9767057 PMCID:PMC88898
- da Silva KE, Maciel WG, Sacchi FPC, Carvalhaes CG, Rodrigues-Costa F, da Silva ACR, Croda MG, Negrão FJ, Croda J, Gales AC, Simionatto S. Risk factors for KPC-producing *Klebsiella pneumoniae* out for surgery. *J Medical Microbiol*. 2016; 65:547-53. <https://doi.org/10.1099/jmm.0.000254> PMID:27002853
- World Health Organization. Antimicrobial resistance global report on surveillance. Geneva: Switzerland: WHO Press. 2014.
- Paczosa MK, Meccas J. *Klebsiella pneumoniae*: going on the offense with a strong defense. *Microbiol Mol Biol Rev*. 2016; 80(3):629-61. <https://doi.org/10.1128/MMBR.00078-15> PMID:27307579 PMCID:PMC4981674
- Heidary M, Nasiri MJ, Dabiri H, Tarashi S. Prevalence of drug-resistant *Klebsiella pneumoniae* in Iran: a review article. *Iran J Public Health*. 2018; 47(3):317-26.
- Sakthivel M, Ayyasamy PM, Prasanth A. Identification and antimicrobial susceptibility testing of pathogenic micro-organism from dental patients. *Asian J Pharm Clin Res*. 2016; 9(2):226-30. <https://doi.org/10.22159/ajpcr.2016.v9s2.13668>
- Garbati MA, Al Godhair AI. The growing resistance of *Klebsiella pneumoniae*; the need to expand our antibiogram: case report and review of the literature. *Afr J Infect Dis*. 2013; 7(1):8-10. <https://doi.org/10.4314/ajid.v7i1.2>

9. Sheth KV, Patel TK, Tripathi CB. Antibiotic sensitivity pattern in neonatal intensive care unit of a tertiary care hospital of India. *Asian J Pharm Clin Res.* 2012; 5(3):46-50.
10. Lagamayo EN. Antimicrobial resistance in major pathogens of hospital-acquired pneumonia in Asian countries. *Am J Infect Control.* 2008; 36(4):101-8. <https://doi.org/10.1016/j.ajic.2007.10.020> PMID:18468549
11. Khan DA, Taj MK, Rehman FU, Mustafa MZ, Taj I, Muhammad G. Isolation and identification of *Klebsiella pneumoniae* causal-agent of pneumoniae from urine of children in hospitals of Quetta city. *J Bio Env Sci.* 2016; 9(4):207-12.
12. Clinical and Laboratory Standard Institute. Performance Standards for Antimicrobial Susceptibility Testing; 26th informational supplement. CLSI document M100-S. Wayne, PA., 2016.
13. Patel SS, Chauhan HC, Patel AC, Shrimali MD, Patel KB, Prajapati BI, et al. Isolation and identification of *Klebsiella pneumoniae* from sheep-case report. *Int J Curr Microbiol App Sci.* 2017; 6(5):331-4. <https://doi.org/10.20546/ijcmas.2017.605.037>
14. Abdullah SM, Zghair ZR. Isolation of *Klebsiella pneumoniae* from urine of human and cattle in Baghdad city with histopathological study experimentally in mice. *Int J Adv Res Biol Sci.* 2016; 3(10):38-45. <https://doi.org/10.22192/ijarbs.2016.03.10.006>
15. Ravichitra KN, Prakash PH, Subbarayudu S, Rao US. Isolation and antibiotics sensitivity of *Klebsiella pneumoniae* from pus, sputum, and urine samples. *Int J Curr Microbiol App Sci.* 2014; 3(3):115-9.
16. Pitout JD, Laupland KB. Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern. *Lancet Infect Dis.* 2008; 8:159-66. [https://doi.org/10.1016/S1473-3099\(08\)70041-0](https://doi.org/10.1016/S1473-3099(08)70041-0)
17. Livermore DM. Beta-lactamase the threat renews. *Curr Protein Pept Sci.* 2009; 10:397-400. <https://doi.org/10.2174/138920309789351994> PMID:19538149
18. Refdinita A, Maksun R, Nurgani A, Endang P. Susceptibility pattern of bacteria to antibiotics in intensive care unit of Fatmawati Hospital Jakarta in 2001-2002. *Makara Kesehatan.* 2004; 8(2):41-8. <https://doi.org/10.7454/msk.v8i2.293>
19. Simanjuntak E. Identification and susceptibility pattern of isolated bacteria from patients with infected urinary tract in Haji Adam Malik General Hospital, Medan. Thesis of Faculty of Medicine, Universitas Sumatera Utara. Medan: Indonesia, 2014.
20. Biswas T, Houghton JL, Garneau-Tsodikova S, Tsodikov OV. The structural basis for substrate versatility of chloramphenicol acetyltransferase CATI. *Protein Sci.* 2012; 21:520-30. <https://doi.org/10.1002/pro.2036> PMID:22294317 PMID:PMC3375752
21. Schwalbe R, Steele-Moore L, Goodwin AC. Antimicrobial susceptibility testing protocols. Boca Raton: CRC Press; 2007:25. <https://doi.org/10.1201/9781420014495>
22. Redgrave LS, Sutton SB, Webber MA, Piddock LJV. Fluoroquinolone resistance: mechanism impact on bacteria, and role in evolutionary success. *Cell Press.* 2014; 22(8):438-45. <https://doi.org/10.1016/j.tim.2014.04.007> PMID:24842194
23. Kumar AR, Kalpana S. Prevalence and antimicrobial susceptibility pattern of *Klebsiella pneumoniae* causing urinary tract infection and issue related to the rational selection of antimicrobial. *Sch J App Med Sci.* 2013; 1(15):395-9.
24. Sirkawar AS, Batra HV. Prevalence of antimicrobial drug resistance of *Klebsiella pneumoniae* in India. *Int J Bioscienc Biochem Biofarma.* 2001; 1(3):211-5. <https://doi.org/10.7763/IJBBB.2011.V1.38>
25. Giedraitienė A, Vitkauskienė A, Naginienė R, Pavilonis A. Antibiotic resistance mechanisms of clinically important bacteria. *Medicina (Kaunas).* 2011; 47(3):137-46. <https://doi.org/10.3390/medicina47030019>
26. Anderl JN, Franklin MJ, Stewart PS. Role of antibiotic penetration limitation in *Klebsiella pneumoniae* biofilm resistance to ampicillin and ciprofloxacin. *Antimicrob Agents Chemother.* 2000; 44:1818-24. <https://doi.org/10.1128/AAC.44.7.1818-1824.2000> PMID:10858336 PMID:PMC89967
27. Bellifa S, Hassaine H, Balestrino D, Charbonnel N, M'hamedi I, Terki IK, et al. Evaluation of biofilm formation of *K. pneumoniae* isolated from medical devices at the University Hospital of Tlemcen, Algeria. *Afr J Microbiol Res.* 2013; 7:5558-64. <https://doi.org/10.5897/AJMR12.2331>
28. Ruiz J. Mechanisms of resistance to quinolones: target alterations, decreased accumulation and DNA gyrase protection. *J Antimicrob Chemother.* 2003; 51(5):1109-17. <https://doi.org/10.1093/jac/dkg222> PMID:12697644
29. Hooper DC, Jacoby GA. Mechanism of drug resistance: quinolone resistance. *Ann N Y Acad Sci.* 2015; 1354(1):12-31. <https://doi.org/10.1111/nyas.12830> PMID:26190223 PMID:PMC4626314
30. Colodner R, Raz R, Chazan B, Sakran W. Susceptibility pattern of ESBL-producing bacteria isolated from inpatients to five antimicrobial drugs in a community hospital in northern Israel. *Int J Antimicrob Agents.* 2004; 24:409-10. <https://doi.org/10.1016/j.ijantimicag.2004.06.001> PMID:15380271
31. Nordmann P, Cuzon G, Naas T. The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria. *Lancet Infect Dis.* 2009; 9:228-36. [https://doi.org/10.1016/S1473-3099\(09\)70054-4](https://doi.org/10.1016/S1473-3099(09)70054-4)
32. Xiao Y, Hu Y. The major aminoglycoside-modifying enzyme AAC (3)-II found in *E. coli* determine a significant disparity in its resistance to gentamicin and amikacin. *Microb Drug Resist.* 2012; 18(1):42-6. <https://doi.org/10.1089/mdr.2010.0190> PMID:22066787