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

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Keywords: Klebsiella pneumonia; Antibiotics; Pus specimens; Susceptibility testing

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Abstract

AIM: This study was to determine the sensitivity of *Klebsiella pneumonia* 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. Pneumonia* 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-aquired 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 susceptibilty of *K. Pneumonia* 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-Proskauermedia, and Mueller Hinton agar (MHA) were obtained from Oxoid (Hampshire, UK). Paper discs containing standard antibiotics namely ampicillin 10 µg, amoxycillin 25 µg, chloramphenicol 30 µg, cefuroxyme 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 (chrystal violet, 96% ethanol, iodin, safranin O, ammonium oxalate, oksalat, para-dimethylaminobenzaldehyde, butanol, acid chloride, α-naphtol 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.

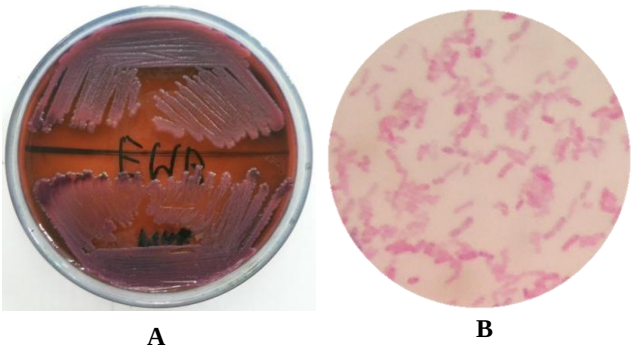
Antibiotic susceptibility testing of bacterial isolates

The specimens were aseptically transferred into brain heart infussion, cultured on eosin metil 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 spesific for *K. pneumoniae*. A total of 20 *K. pneumoniae* isolates were obtained in this study.



A B

Figure 1: Characteristics of Klebsiella pneumoniae on eosin methylen 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'sName	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%)
Fluoroquinolone	Levofloxacin	12 (60.0%)	1 (5.0%)	7 (35.0%)
	Ciprofloxacin	11 (55.0%)	1 (5.0%)	8 (40.0%)
Drug Combination	Co-trimoxazole	10 (50.0%)	0 (0.0%)	10 (50.0%)
Cephalosporin	Ceftazidime	11 (55.0%)	2 (10.0%)	7 (35.0%)
	Cefoperazone	10 (50.0%)	5 (25.0%)	5 (25.0%)
	Cefuroxyme	9 (45.0%)	2 (10.0%)	9 (45.0%)
	Cefepime	7 (35.0%)	7 (35.0%)	6 (30.0%)
	Cefotaxime	6 (30.0%)	1 (5.0%)	13 (65.0%)
Chloramphenicol	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 sensivity, but the emergence of bacterial resistance to antibiotics tested also detected.

Ampicillin and amoxicillin which are classified into penicilline derivates 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 amoxyclav and ofloxacin [15]. Penicillin resistance is due to the ability of *K. pneumoniae* to carry plasmids producing beta-lactamse 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. pneumonia* 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. pneumonia* producing extended spectrum beta-lactamase [30]. However, the bacterial resistance to carbapenem is possible since *K. pneumonia* 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 enzymesleading many bacteria are sensitive to this antibiotic [32].

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

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